



Eastern Pilbara Ghost Bat Monitoring Program: 2022-2023

Report to BHP WAIO

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Executive Summary

BHP Western Australian Iron Ore (BHP WAIO) commissioned Biologic Environmental Survey (Biologic) to undertake a monitoring program for ghost bats (*Macroderma gigas*) within the Eastern Pilbara region, specifically at Western Ridge and Jimblebar (including Ninga), as well as in Cathedral Gorge (hereafter referred to as the Study Area). The objectives of the ghost bat monitoring program are to understand the population dynamics, cave usage and significance, and ghost bat movements in the Eastern Pilbara region (with a particular focus on Western Ridge and Jimblebar). This monitoring report covers work undertaken between February and September 2023.

Forty-one (41) caves were monitored over 145 visitation events between January and September 2023. Ghost bat presence within caves was determined using four different survey techniques: direct observation, scat observations, ultrasonic recordings, and motion cameras. Ghost bat usage of the caves is determined from the scat monitoring, hormone analysis, genetic analysis and motion cameras. Additionally, dietary analysis, microclimate monitoring and GPS tracking were also used to provide insight into how bats use their environment.

Eleven (11) unique individuals (genotypes), three females and eight males, were identified from six caves at Western Ridge and Jimblebar during 2023. The most individuals were recorded from CWER-01 (3 total: 1F; 2M) and CWER-03 (4 total: 2F; 2M) and CNIN-03 (2M). The remaining three caves only recorded one individual each, which were all males. Four of the six caves contained scat with elevated progesterone levels (CNIN-01, CNIN-03, CWER-01 and CWER-03), which were all classified as high levels (greater than 1,000ng/g). There was no significant change in the genetic health of the ghost bat population from 2022 to 2023, the inbreeding coefficient improved slightly in 2023, resulting in a slightly higher effective population size in 2023 of 11.9 individuals compared with 6.4 individuals in 2022.

Ghost bats were recorded via ultrasonic recorder at six of the eight monitored roosts: CWER-01, CWER-03, CWER-16, CJIM-05, CNIN-03 and CNIN-09. The highest number of calls across the monitoring period were generally in May and June 2023. In May 2023, calls were recorded at four caves (CWER-01, CWER-16, CNIN-03 and CNIN-09), with the timing of calls indicated that roosting was occurring in three caves (CWER-16, CNIN-03 and CNIN-09).

The mean temperature within the monitoring caves ranged from 25.51°C (at CWER-14) to 31.4°C (at CJIM-04). Category 4 caves tended to have fewer temperature recordings within the preferred range. Only two of the 13 Category 3 caves and none of the Category 2 caves were in the preferred range for less than 30% of the monitoring period. The mean relative humidity recorded at the monitored roosts ranged from 24.26% (at CCAT-17) to 61.77% (at CJIM-09).

It is logical to assume that caves with a high number of scats are more important to ghost bats. Based on this, the caves that have had the highest deposition during the monitoring period (limited to the sheets where timeframes can be determined) were CWER-03 and CNIN-01 in 2023 and CWER-03 in 2022. However, the number of scats varied in these caves between trips and years. This pattern of fluctuating cave use (determined via scat deposition) is consistent with other regions of the Pilbara (especially the area around South Flank/Mining Area C where most work to date has been done) and may be influenced by a number of factors including the availability of food resources and possible changes to cave temperatures.

Two caves, CNIN-01 and CWER-16, warrant consideration for changes in status from Category 3 to Category 2, though further data for both caves will be required to verify this. Although individuals in the Study Area currently appear to be stable, due to the inherently low numbers and sparse distribution, the future stability of this population needs monitoring during mining activities to avoid significant impacts. As of the 30th June 2023, approximately 7,293.64 ha of land associated with active mining had been cleared in the Study Area. Ten monitoring caves (including CWER-04 that recorded ghost bats) are located within less than 1 km of mining disturbance (i.e. active mining).

1 Introduction

1.1 Background

BHP Western Australian Iron Ore (BHP WAIO) commissioned Biologic Environmental Survey (Biologic) to undertake a monitoring program for ghost bats (*Macroderma gigas*) within the Eastern Pilbara region, specifically at Western Ridge and Jimblebar (including Ninga), as well as in Cathedral Gorge (hereafter referred to as the Study Area). The Study Area is not defined by any discreet boundaries, but generally encompasses the Eastern Pilbara area south-west, north-west and east of Newman.

The ghost bat, listed as Vulnerable under the *Environment Protection and Biodiversity Conservation Act 1999* (EPBC Act) and *Biodiversity Conservation Act 2016* (BC Act) (Appendix A), is known to occur in the Study Area.

Commencing in 2021, BHP WAIO established a ghost bat monitoring program across the Eastern Pilbara. The program was designed to provide baseline information to better understand ghost bats in the area and inform future environmental management within this area. Monitoring of ghost bats in the caves surrounding the Jimblebar mine was a condition of its approval.

1.2 Objectives

The objectives of the ghost bat monitoring program are to understand the population dynamics, cave usage and significance, and ghost bat movements in the Eastern Pilbara region (with a particular focus on Western Ridge and Jimblebar). This monitoring report covers work undertaken between February and September 2023 and includes the sixth to ninth monitoring surveys in the Study Area.

2 Monitoring Methods

Monitoring was undertaken in two focus areas, Western Ridge and Jimblebar (including Ninga) as well as at Cathedral Gorge (Figure 2.2). The monitoring continues the methods and approaches commenced in September 2021 (refer to Appendix B for detail on the standard monitoring methods).

2.1 Monitoring Timing, Personnel and Licences

Monitoring was conducted over four trips (Table 2.1). The monitoring surveys were undertaken by experienced zoologists Chris Knuckey (Principal Zoologist), Hannah Anderson (Senior Zoologist), Stephen McGrath (Zoologist), Georgina Mattner (Zoologist), Aleesha Turner (Zoologist) and Sammy Alatas (Zoologist) whom collectively have over 40 years of experience undertaking relevant surveys and monitoring within the Pilbara region, including targeted surveys for the ghost bat (Appendix C).

To minimise disturbance of the breeding cycle of the population, surveys were scheduled to avoid the later stages of the reproduction period, when female ghost bats are most likely to be heavily pregnant and/or lactating (October to December (Bat Call, 2021)).

Note, the first monitoring survey within this report (Trip 6) collected data from monitoring methods established in the previous years' monitoring, and so initial data collected in Trip 6 dates back to September 2022. Hence, the sampling period discussed within this report extends between September 2022 to September 2023.

Table 2.1: Monitoring surveys and personnel

Trip	Duration	Personnel
6	30 th January – 3 rd February 2023	Chris Knuckey, Stephen McGrath, Georgina Mattner
7	20 th – 24 th April 2023	Chris Knuckey, Stephen McGrath, Aleesha Turner
8	29 th June – 3 rd July 2023	Chris Knuckey, Stephen McGrath
9	8 th – 12 th September 2023	Chris Knuckey, Hannah Anderson, Sammy Alatas

Sampling was conducted under the *Animal Welfare Act 2002* licence to use animals for scientific purposes (License No. U244/2022-2024), administered through the Department of Primary Industries and Regional Development (DPIRD). This was facilitated by Murdoch University's Animal Ethics Committee (AEC) (Permit RW3354/21).

Under Section 40 of the BC Act, threatened species sampling was completed under a Department of Biodiversity, Conservation and Attractions (DBCA) "Authorisation to Take or Disturb Threatened Species", issued to Chris Knuckey (authorisation number TFA 2223-0123).

2.2 Weather

Weather recorded for the sampling period (September 2022 to September 2023) was obtained from Newman Aero Station (station number 007176), approximately 15 km south of the Study Area. Over the 12 month monitoring period, 275 millimetres (mm) of rainfall was recorded, which is lower than the long-term annual average (LTA) rainfall for the same period (371.6 mm) (BoM, 2023) (Figure 2.1). Below average rainfall was also recorded in the 12 months prior to the survey, with only 286 mm of rainfall recorded. Observed minimum and maximum monthly temperatures were comparable to the long-term annual average temperatures for the same period (Figure 2.1).

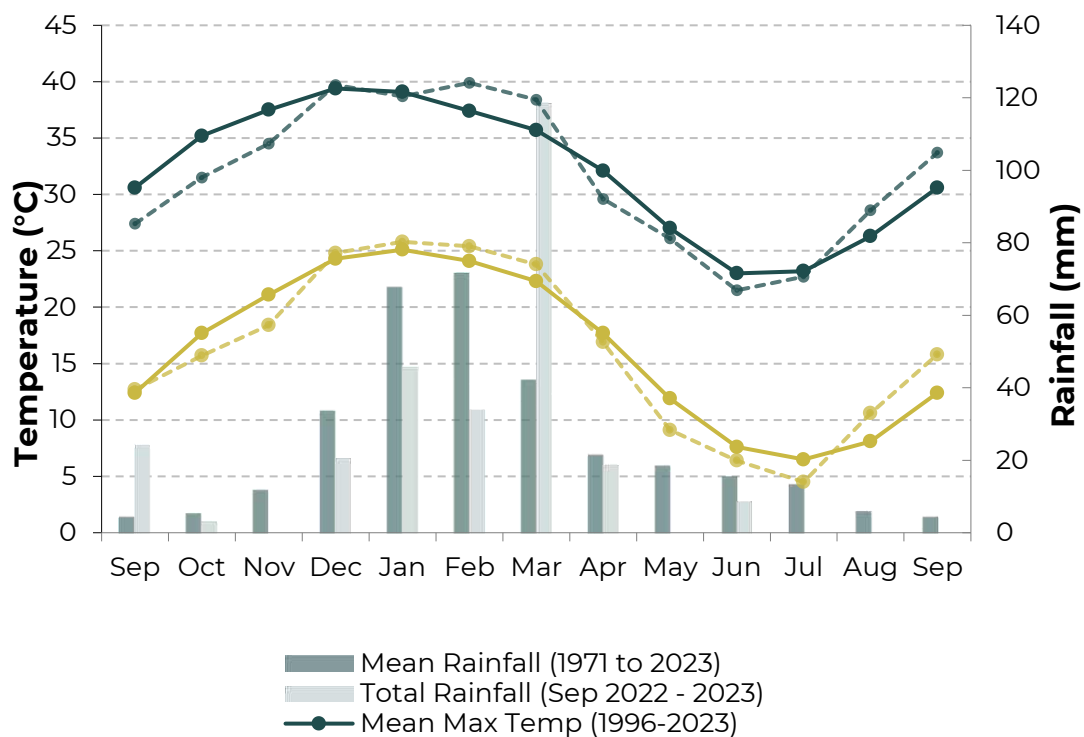


Figure 2.1: Recent (2022-2023) and long-term average climate data for Newman Aero (BoM, 2023)

2.3 Monitoring Locations

Forty-one (41) caves were monitored over 145 visitation events between January and September 2023 (Figure 2.2; and see Appendix D). CJIM-20 was not able to be accessed between September and June 2023 (Trip 8). This cave was also not able to be accessed during the June and September 2022 monitoring due to access issues.

CNIN-05 was visited in January 2023 when a cave assessment was undertaken, but this cave is currently not part of the monitoring program.

2.4 Scat Counts and Collection

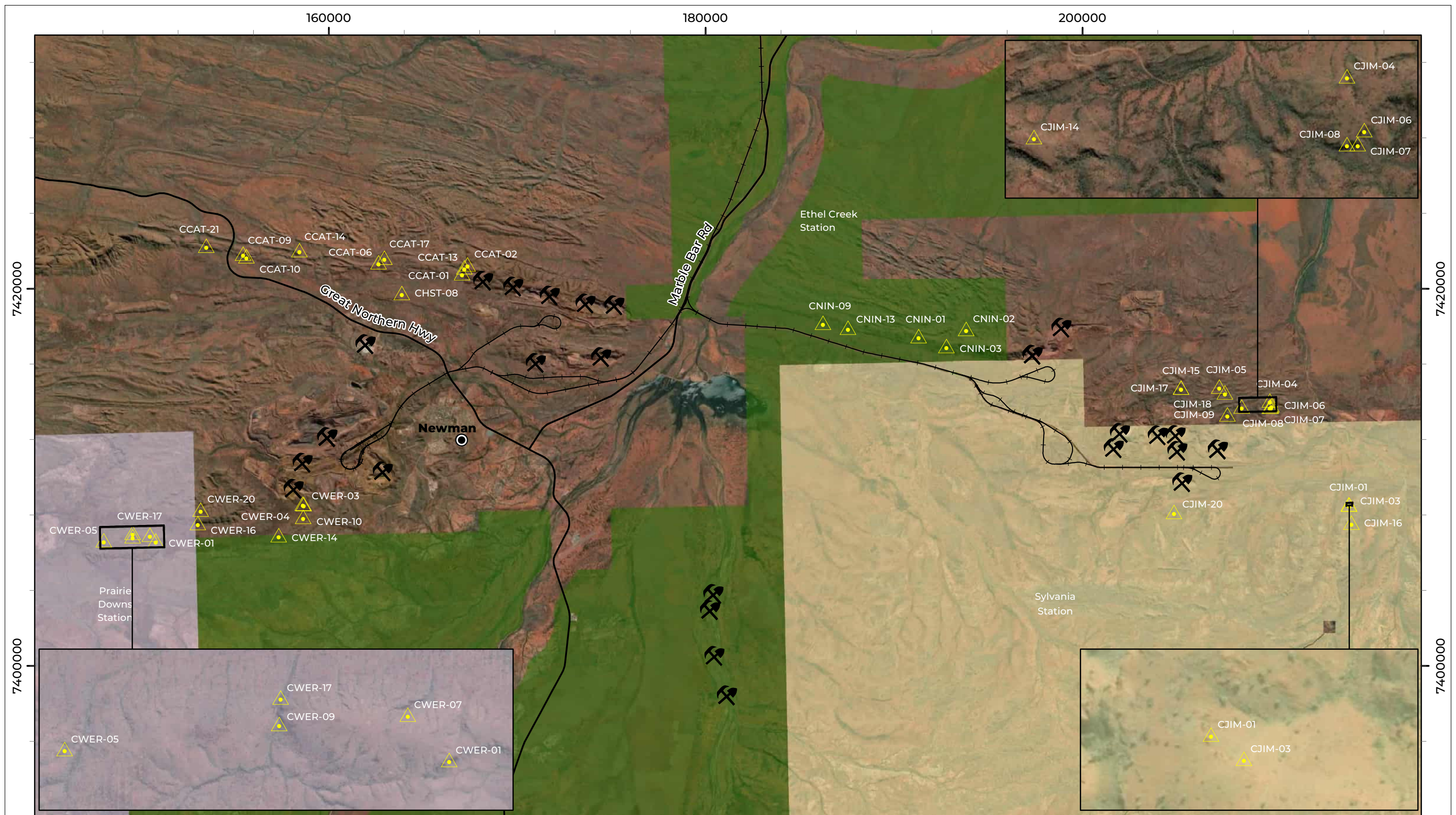
Scat monitoring was undertaken at 38 caves. Access to CNIN-09 and CWER-16 was restricted due to health and safety reasons. Fresh scats (generally between 1-20 and a maximum of 100 scats) were collected from the sheets during each visit (Table 2.2). Scats collected in January 2023 were deposited after September 2022 (Trip 5 of the prior year's monitoring).

In total, 474 scats were collected from six caves at Western Ridge and Jimblebar (Figure 2.2). No scats were deposited on sheets at the Cathedral Gorge monitoring sites.

Table 2.2: Number of scats collected during each monitoring survey

Area	Cave ID	Trip 6 Jan/Feb 2023	Trip 7 Apr 2023	Trip 8 Jun/Jul 2023	Trip 9 Sep 2023	Total Scats Collected
Western Ridge	CWER-01	0	0	8	0	8
	CWER-03	71	0	1	0	72
	CWER-17	0	0	8	15	23
Jimblebar/ Ninga	CNIN-01	0	5 [^]	221 [*]	101	327
	CNIN-03	2	0	0	10	12
	CNIN-13	0	0	12	20	32
Total		73	5	250	146	474

[^] denotes scats collected but not from black scat sheets; ^{*} 71 for genetics, 150 for dietary analysis



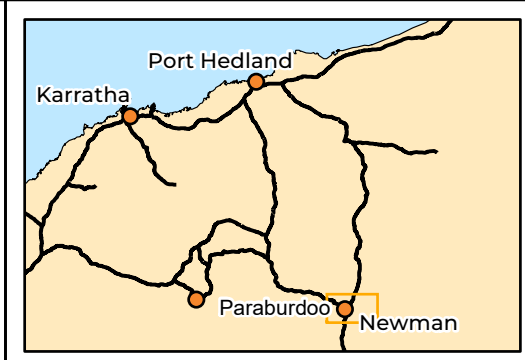
- LEGEND**
- Monitoring Cave
 - Operating Mine
 - State Road
 - Rail
 - Pastoral Lease
 - Ethel Creek Station
 - Prairie Downs Station
 - Sylvania Station

Biologic

Scale 1:184,000

0 3 6 Km

Coordinate System: GDA 1994 MGA Zone 51
Projection: Transverse Mercator
Datum: GDA 1994
Created 04/09/2024



BHP WAIO
Eastern Pilbara
Ghost Bat Monitoring
2022-2023

Figure 2.2: Monitoring locations

2.5 Hormone Analysis

Of the 474 scats collected, 324 samples from six caves were analysed by Dr Tamara Keeley from the University of Queensland for faecal metabolites (hormones) to determine the occurrence of pregnant individuals within caves. This method has been used for numerous other bat species (Keeley *et al.*, 2012a; Keeley *et al.*, 2012b) and was validated for ghost bat in a pilot study using the captive breeding colony from Perth Zoo (Keeley *et al.*, 2023).

The hormone analysis aids in determining if breeding is occurring within the Study Area. This, in combination with results from genetic analysis of scats which can identify the presence and abundance of specific pregnant individuals at each cave, were utilised to identify the presence of potential maternity roosts.

2.6 Genetic Analysis

Of the 474 scats collected, 319 samples from six caves, were analysed by Diana Prada, Melissa Millar and Kym Ottewell from DBCA (an additional five were sent but not analysed due to being of insufficient size). An additional 22 samples collected in December 2019 (prior to the monitoring program) were added to the analysis to supplement the dataset, and are included in the results and discussion, despite being collected outside the 2023 monitoring period.

The average amplification success rate was 77%, including replicates, so the filtered dataset for analysis totalled 239 samples that were amplified at 40 loci. Due to the variable quality of scat samples, moderately conservative filtering thresholds were used to determine unique individuals (Appendix B). Results from the current monitoring period were compared with genetic data from scat previously genotyped by DBCA to determine movement of individuals over temporal and spatial scales. Genetic diversity indices were determined from SNP markers of 11 detected individuals from both 2023 and 2022. Two alleles were used for each cohort. Observed heterozygosity (H_o); unbiased expected heterozygosity (uH_e); inbreeding coefficient (F) and effective population size (N_e) were determined (Appendix B).

2.7 Dietary Analysis

One hundred and fifty (150) scats collected from CNIN-01 in September 2023 were sent to Georgeanna Story from Scats About for dietary analysis. Three of the scats were too degraded to be analysed, so 147 scats were used for analysis.

2.8 Ultrasonic Analysis

SongMeter SM4BAT-FS bat detectors (SM4; Wildlife Acoustics, USA) powered by an external solar power supply were previously installed at eight caves in 2022. A total of 2,582 recording nights between September 2022 and September 2023 were analysed (Table 2.3; Figure 2.2).

Monitoring of CCAT-06 ended in February 2023 due to a lack of ghost bat records during the previous two years of monitoring. This cave was replaced by CWER-16 in February 2023 (Table 2.3).

Table 2.3: Location and deployment dates of SongMeters

Area	Cave ID	Start	Finish	Total Nights
Western Ridge	CWER-01	Sept 2022	Ongoing	367
	CWER-03	Sept 2022	Ongoing	367
	CWER-16	Feb 2023	Ongoing	217
Jimblebar/ Ninga	CJIM-03	Sept 2022	Ongoing	371
	CJIM-05	Sept 2022	Ongoing	369
	CNIN-03	Sept 2022	Ongoing	370
	CNIN-09	Sept 2022	Ongoing	370
Cathedral Gorge	CCAT-06	Sept 2022	Jan 2023	151
			Total	2,582

Analysis of audio and ultrasonic calls was undertaken by Robert Bullen of Bat Call WA using standardised techniques and calls for Pilbara leaf-nosed bat (*Rhinonicteris aurantia* 'Pilbara form') were also included. Raw files were first scanned for ghost bat and Pilbara leaf-nosed bat calls using Kaleidoscope software (Wildlife Acoustics, USA) and referenced against Bat Call WA's personal call library, then reviewed for significant times and call numbers using Cool Edit software (Adobe, USA). The total number of calls and the time of the first and last call for each sampling night were recorded. A sampling night was from sunset to sunrise the following day. As bats may emit multiple calls while using a site, recorded calls were used to indicate the presence of bats rather the number of individuals which might be present. Quiet calls may be difficult to distinguish from other noise or the calls of other bat species, and a ghost bat may use a site without calling at all (instead navigating by sight alone); thus, an absence of calls does not always indicate an absence of ghost bats. When multiple calls were recorded simultaneously, or when calling was constantly occurring during a sampling night, the number of calls were not counted, with a note of 'multiple calls' only being recorded instead.

The timing of calls recorded at a given site may reveal whether individuals used the site as a nocturnal roost (i.e. visited briefly during the night during foraging or dispersal activity) or a

diurnal roost. Calls recorded immediately after sunset (i.e. when ghost bats typically depart roosts to commence foraging) are presumed to be by individuals who roosted in the site during the day, while calls recorded soon before sunrise are presumed to be by individuals returning to the roost after a night of foraging. Following Bat Call WA's analysis methodology, diurnal roosting is considered to be occurring when the last call during the previous recording night occurred after dawn or ≤ 10 minutes before dawn; or when the first call during the current monitoring night was within ≤ 10 minutes of dusk. During these time periods, if a call was emitted it was assumed that one call equates to one individual, and the number of individuals diurnally roosting was used (rather than the number of calls that is used for overall bat presence, including nocturnal roosting).

2.9 Camera Monitoring

Infrared-lit motion surveillance cameras set to record videos, were monitoring seven caves between September 2022 and September 2023 (six in 2022 and one additional cave in 2023). A total of 2,351 nights were recorded (Table 2.4).

Due to the volume of data recorded, review of camera footage was only undertaken on dates where bats were recorded on the ultrasonic detectors, to verify these results and provide further information on visitations (such as the number of bats present and the activities taking place; night roosting, day roosting, feeding etc.). At CWER-17, where there was no ultrasonic detector installed, footage was checked on one randomly selected night each week.

Table 2.4: Camera trap locations

Area	Cave ID	Total Nights	Monitoring Dates	Comments
Western Ridge	CWER-01	367	7/09/22 - 9/09/23	This cave has more than one chamber, it is possible all ghost bat visitations are not captured as individuals may utilise different chambers.
	CWER-03	366	8/09/22 - 9/09/23	-
	CWER-17	138	23/04/23 - 8/09/23	-
Jimblebar/ Ninga	CJIM-03	371	6/09/22 - 12/09/23	-
	CJIM-05	369	6/09/22 - 10/09/23	The roosting point area of this cave is quite high and so the camera is pointed directly upright – this means bats are harder to identify when flying by. Dust also builds up on the lens causing poorer visibility over time.
	CNIN-03	370	6/09/22 - 11/09/23	This cave has more than one chamber, it is possible all ghost bat visitations are not captured as individuals may utilise different chambers. Camera was knocked over by a wallaby, so some data was lost between December 2022 and January 2023.
	CNIN-09	370	6/09/22 - 11/09/23	Due to safety access concerns, the camera is placed outside of the cave. This creates issues with spiderwebs which leads to large amounts of false triggers and poor visibility.

2.10 Microclimate Analysis

Microclimate loggers (HOBO (MX2301A) temperature/ RH Bluetooth data loggers) were installed at 38 caves to assess the interior microclimate (temperature and relative humidity [RH]). Loggers were not able to be installed at CWER-16 and CNIN-09 due to safety restrictions.

The microclimate loggers were deployed as close as possible to the likely roosting location within each cave. All loggers recorded at standardised 3-hour intervals.

At each roost, the range of temperature recorded (records of daily minimum and maximum parameters) was plotted against the range typical of a Category 2 or 3 ghost bat roost (i.e. 28-32°C, Bat Call, 2021).

2.11 GPS Tracking

Trapping of ghost bats for GPS tracking was attempted at four caves (Table 2.5). Six trapping attempts were conducted. Trapping was confined to caves with a reasonable chance of individuals being present, those caves being Category 2 caves and a small selection of Category 3 caves. However, because the roosting chamber for many of these caves was under surveillance by camera monitoring (Section 0), no trapping was completed if it could be confirmed no ghost bats were present at the time of survey.

No ghost bats were utilising the roosts at the time when trapping occurred.

Table 2.5: Caves trapped for ghost bat

Area	Cave ID	No. Events	Trip 6	Trip 7	Trip 8	Trip 9
Western Ridge	CWER-01	1	2/02/2023	-	-	-
Jimblebar/ Ninga	CNIN-03	3	2/02/2023	21/04/2023	-	11/09/2023
	CNIN-09	1	-	-	-	11/09/2023
	CNIN-13	1	-	-	-	11/09/2023

2.12 Habitat and Disturbance Monitoring

Photo monitoring was undertaken at 37 caves monitored. Three of the caves (CCAT-10, CCAT-13 and CCAT-21) were not subject to photo monitoring in this monitoring period as they were not visited on trip 9, when photo monitoring was completed.

Disturbance layers were provided by BHP for the period up to 30th June 2023 and distance between disturbance and monitoring cave determined.

2.13 Monitoring Constraints and Limitations

The following constraints are applicable to this monitoring period (Appendix D: Table 5). The below considers those aspects outlined by the EPA (2020), noting the technical guidance is specific to fauna surveys and not monitoring programs.

A number of issues were encountered with the microclimate loggers:

- In July 2023, the logger in CNIN-03 was unable to be located, presumably moved by an animal, a replacement logger was installed but a period of data was lost.
- CJIM-09 was not visited in January 2023 (Trip 6), and when it was visited in April 2023 the battery was found to be dead. Prior to this, it was last visited in September 2022, when the data was last downloaded. The battery was replaced in April 2023, and so data is only available after this time.

- In April 2023 the logger at CCAT-10 was found to have been moved outside the cave (presumably by animals) and was non-functional, presumably damaged from exposure to rain. There was no data available from this logger.
- A number of other caves also suffered from data loss due to various technical issues (mostly with battery health and the corruption of data).

Some of the infra-red cameras were knocked by animals (CNIN-03), and spider-webs covered some portion of the lenses (CNIN-09) (Table 2.4; Appendix D: Table 5).

None of these constraints were considered significant to the outcomes of the monitoring program.

No ghost bats were utilising the roosts at the time when trapping attempts occurred, therefore no ghost bats were tagged for monitoring in 2023.

3 Monitoring Results

3.1 Ghost Bat Observations

One alive individual was observed during Trip 8 in the rear chamber of CNIN-13 on 2nd July 2023. A further 25 observations of ghost bats were recorded via camera from six caves (CWER-01, CWER-03, CWER-17, CJIM-05, CNIN-03 and CNIN-09) (Section 3.7; Appendix I).

3.2 Scat Monitoring

Scats were recorded on sheets at six of the 38 caves within which scat monitoring was undertaken (Table 3.1; Appendix E). Five scats were also opportunistically collected from the cave floor in CNIN-01.

For the caves where scats were recorded, the total number of scats during a monitoring period ranged from 8 to 230 (Table 3.1). The largest number of scats in a monitoring period were recorded in CNIN-01 (230 scats/1.83 per day average, deposited between April and June) and CWER-03 (200 scats/ 0.92 per day average, deposited between September 2022 and January 2023). The largest number of scats was recorded from CNIN-01; 380 scats deposited between April and September 2023. With the exception of CWER-03 that had 200 scats deposited during one period, the remainder of the caves all had less than 100 scats deposited during the 12 months of monitoring.

Table 3.1: Scat deposition from monitored caves during monitoring period

Area	Cave ID	Trip 6 Jan/Feb 2023	Trip 7 Apr 2023	Trip 8 Jun/Jul 2023	Trip 9 Sep 2023	Total (Av.)
Western Ridge	CWER-01	0	0	8 (0.34)	0	8 (0.15)
	CWER-03	200 (0.92)	0	1 (0.12)	0	201 (0.66)
	CWER-17	0	0	8 (0.34)	15 (0.46)	23 (0.25)
Jimblebar/Ninga	CNIN-01	0	0	230 (1.83)	150 (1.45)	380 (1.01)
	CNIN-03	2 (0.12)	0	0	10 (0.38)	12 (0.18)
	CNIN-13	0	0	12 (0.41)	50 (0.84)	62 (0.41)
Total/ average		202 (0.58)	0	259 (0.61)	225 (0.78)	686

3.3 Hormone Analysis

Four of the six caves contained scat with elevated progesterone levels (CNIN-01, CNIN-03, CWER-01 and CWER-03), which were all classified as high levels (greater than 1,000ng/g) (Appendix F). Two of these caves (CWER-01 and CWER-03) also had intermediate levels of progesterone (between 700 and 999ng/g) (Appendix F). Elevated progesterone levels were collected during three of the four sampling events (Table 3.2).

Table 3.2: Number of samples with elevated (H) or intermediate (I) progesterone as a portion of the total number of scats (#) analysed

Cave ID	Trip 6 Jan/Feb			Trip 7 Apr			Trip 8 Jun/Jul			Trip 9 Sep			Total (H&I)
	#	H	I	#	H	I	#	H	I	#	H	I	
CWER-01	0	-	-	0	-	-	8	4	3	0	-	-	7
CWER-03	71	5	10	0	-	-	1	0	0	0	-	-	16
CWER-17	0	-	-	0	-	-	8	0	0	15	0	0	0
CNIN-01	0	-	-	5	0	0	71	3	0	101	7	0	10
CNIN-03	2	1	0	0	-	-	0	-	-	10	2	1	4
CNIN-13	0	-	-	0	-	-	12	4	3	20	-	-	7
Total	73	7	10	5	0	0	100	11	6	146	9	1	44

Note: # = total number of scats analysed; 'H' = elevated levels of progesterone (>1,000 ng/g); 'I' = intermediate levels of progesterone (700-999 ng/g)

3.4 Genetic Analysis

These results are summarised from the report provided by DBCA. The full report is provided in Appendix G. Eleven (11) unique individuals (genotypes), three females and eight males, were identified from six caves at Western Ridge and Jimblebar/Ninga during 2023 (Table 3.3).

The most individuals were recorded from CWER-01 (3 total: 1F; 2M) and CWER-03 (4 total: 2F; 2M) and CNIN-03 (2M). Three individuals were identified from the 2019 samples collected in CWER-03. The remaining three caves only recorded one individual, which were all males. No samples collected from CWER-02 in 2019 were successfully genotyped. This is the first survey where individual genotypes have been recorded for caves CNIN-01 and CNIN-13.

Five individuals (#906 - 909 and 916) were detected for the first time during the monitoring program. Those with identification numbers less than #906 have all been recorded previously. Only one individual, male #906, was recorded at multiple caves (CWER-01 and CWER-17). These caves are approximately 1.3 km apart.

Three individuals (1F; 1M; 1U) were recorded from the 2019 samples (all from CWER-03). This is the first record of individual #909.

Table 3.3: Number of genotyped individuals detected by cave (2023 & Dec 2019)

Area	Cave ID	Total*	# Ind. 2023	Bat ID	Total*	# Ind. 2019	Bat ID
Western Ridge	CWER-01	8/5	3	133F 508M 906M	-	-	-
	CWER-02	-	-	-	2/0	0	-
	CWER-03	72/47	4	856F 873M 874M 877F	20/16	3	133F 135F 909U
	CWER-17	23/18	1	906M	-	-	-
Jimblebar/ Ninga	CNIN-01	172/114	1	908M	-	-	-
	CNIN-03	12/8	2	875M 916M	-	-	-
	CNIN-13	32/31	1	907M			

* Total number sent for analysis/ successfully genotyped. **Bold** indicates detected from more than one cave.

Four males were recorded from different collection dates within the 2023 sampling period and had the highest number of scats collected per individual: #873 (CWER-03, January & June, 33 scats); #906 (CWER-17, June & September, 19 scats); #907 (CNIN-13, July & September, 31 scats); and #908 (CNIN-01, April, July, September, 114 scats). The greatest number of scats recorded from a female bat was 11 (#856), all collected in January 2023.

Of the 13 individuals recorded to date from the Study Area, four females and four males have been recorded from more than one year (Table 3.4). Two of the females (#133 and #135) have been recorded over the longest period, with both being recorded in 2016 and 2019, and again in 2022 (#135) and 2023 (#133). (Note that samples collected prior to this monitoring program were collected as regional samples for an ongoing monitoring program at Southern Flank).

The female #133 is also the individual that has recorded the greatest distance of travel, being recorded at CWER-03 in 2016, and CPUN-02 and PWER-01 in 2017. The distance between the Punda cave and those at Western Ridge is approximately 45 km. She is also the bat that has been recorded most consistently across the data collection period (recorded from six of the seven years for which data are available) and from the greatest number of caves (three; CWER-01, CWER-03 and CPUN-02). Female #135 has been recorded from five of the seven years of available sampling data. The remaining two females (#856 and #877) were both recorded in 2022 and 2023 from single caves (CWER-03).

Males have been recorded in higher numbers (eight individuals), but only three have been recorded from multiple caves; #508, #874, and #906 (Table 3.4). The longest time between records for males is five years (#508 recorded between 2018 and 2023). Six of the males have only been recorded from a single sampling event (all but one from 2023). The only other males to have been recorded over multiple years were those recorded in 2022 and 2023.

Most recapture (repeat) samples (25) were collected from the same cave during the same collection period. There is only one record of travel further than this; the movement of female #133 between Western Ridge and Punda in 2016/2017 (Table 3.4; Appendix G).

Table 3.4: Long-term detection history of recaptures

Bat ID	Cave ID	2016	2017	2018	2019	2020	2022	2023
133F	CWER-01		x	x	x			x
	CWER-03	x			x	x		
	CPUN-02		x					
135F	CWER-01	x	x					
	CWER-03	x			x	x	x	
508M	CWER-01			x	x			x
	CWER-16					x		
856F	CWER-03						x	x
873M	CWER-03						x	x
874M	CWER-03						x	x
	CWER-17						x	
875M	CNIN-03						x	x
877F	CWER-03						x	x
906M	CWER-01							x
	CWER-17							x
907M	CNIN-13							x
908M	CNIN-01							x
909U	CWER-03				x			
916M	CNIN-03							x

The caves that have shown the most consistent use across the available dataset are all at Western Ridge (Table 3.3), although this is because there have been no scats collected for analysis from Jimblebar prior to the monitoring program.

CWER-01 has had ghost bat presence within it between 2016 and 2023, with two females (#133 and #135) and one male (#508) consistently being recorded from the cave over a period of years. These two females have also consistently been recorded in CWER-03, which is located approximately 8 km to the east of CWER-01, between 2016 and 2020.

The only other caves that have been visited over multiple years are CWER-17 and CNIN-03, which both only show evidence of visitation from 2022 and 2023.

Table 3.5: Cave visitation history by individual

Area	Cave ID	2016	2017	2018	2019	2020	2022	2023
Western Ridge	CWER-01		133F	133F	133F			133F
		135F	135F					
				508M	508M			508M
								906M
	CWER-03	133F			133F	133F		
		135F			135F	135F	135F	
							873M	873M
							856F	856F
							874M	874M
							877F	877F
					909U			
	CWER-16					508M		
	CWER-17						874M	
								906M
Jimblebar/ Ninga	CNIN-01							908M
	CNIN-03						875M	875M
								916M
	CNIN-13							907M
Punda	CPUN-02		133F					

There was no significant change in the genetic health of the eastern Pilbara ghost bat population from 2022 to 2023 (Table 3.6). There is a marginal drop in the inbreeding coefficient (F , 0.13 to 0.03). This result is due to a slight increase in the number of heterozygotes (H_o , 0.35 to 0.37). Positive values are an excess of homozygotes, which indicates possible inbreeding. While the result suggests a slight improvement in the inbreeding coefficient, the confidence intervals are overlapping, so not considered significant and may be due to sampling error.

The decrease in inbreeding coefficient also corresponded with an increase in the effective population size (N_e), as more heterozygotes suggests that there are more genetically distinct individuals contributing to the sampled gene pool. While the result suggests a slight improvement in effective population size, the confidence intervals are also overlapping, so not considered significant and may be due to sampling error.

Table 3.6: Genetic diversity estimates from 2022 and 2023 monitoring (Ho = observed heterozygosity; uHe = unbiased expected heterozygosity; F = inbreeding coefficient; Ne = effective population size)

Year	No. Individuals	Ho	uHe	F	Ne (95%CI)
2022	11	0.35 ± 0.03	0.37 ± 0.02	0.03 ± 0.06	6.4 (2.6-18.1)
2023	11	0.37±0.034	0.44 ± 0.01	0.13 ± 0.07	11.9 (5.5 – 37.4)

Only two of the 13 individuals from the Study Area dataset had relatedness values consistent with a full-sibling or parent-offspring relationship: #856(F) and #877 (F). Both individuals were collected from CWER-03 in January 2023. Individual #856 has been recorded in this cave from scats collected in February and November 2022, whilst #877 was also recorded from scats collected in February 2022. Second degree relationships (25% similarity, have one relative between them, i.e. grandparent/grandchild, half-sibling, or aunt/uncle with niece/nephew) were recorded for eight pairs:

- #133 (F) / 135 (F);
- #135 (F) / 856 (F) / 877 (F);
- #508 (M) / 906 (M) / 908 (M)

3.5 Dietary Analysis

Budgerigars (*Melopsittacus undulatus*) were by far the most common prey species in the scats analysed, with almost half (70; 48%) of the 147 scats analysed containing this species (Figure 3.1; Appendix H). When it was present in a scat, it was usually the only species present (60 out of 70 samples comprised 100% budgerigar). Other avian species were present in 36 samples (24%), and again usually comprised the only food source in the scat (32 of 36 samples comprised 100% avian remains) (Figure 3.1; Appendix H).

The introduced house mouse (*Mus musculus*) was the next most common prey species, occurring in 45 samples (31%) (Figure 3.1). The only other mammal identified was from an unidentified dasyurid, where it comprised less than 5% of the sample (Figure 3.1). The rest of the sample contained budgerigar (70%) and house mouse (25%).

Twelve samples (8%) contained invertebrates, comprising beetles (eight samples), moths/butterflies (three samples), and one sample contained 10% unidentified insects. Seeds comprised 20% of one sample, which are almost certainly gut contents from the budgerigars that made up the remaining 80%. Unidentified Dasyurid remains were found in only one sample, where it was only a very small proportion of the sample (5%). This was the only evidence of native mammal in the dataset. There were no reptiles recorded in the samples.

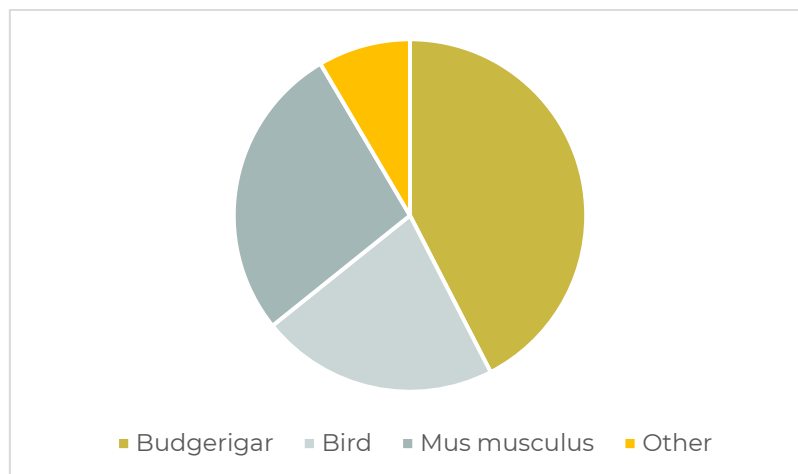


Figure 3.1: Dietary composition of scats analysed from CNIN-01 in September 2023

3.6 Ultrasonic Analysis

Ghost bats were recorded via ultrasonic recorder at six of the eight monitored roosts: CWER-01, CWER-03, CWER-16, CJIM-05, CNIN-03 and CNIN-09 (Appendix K).

The highest number of calls across the monitoring period were generally in May and June 2023. In May, calls were recorded at four caves (CWER-01, CWER-16, CNIN-03 and CNIN-09), with the timing of calls indicated that roosting was occurring in three caves (CWER-16, CNIN-03 and CNIN-09) (Appendix K). Between 27th and 29th May, calls were recorded and based on call patterns it is estimated approximately five individuals were responsible for the calls which were recorded at three separate caves (two in CWER-16, three in CNIN-03 and one in CNIN-09) (Appendix K).

The ultrasonic recorder at CWER-16 was installed in February 2023, with a total of 217 recording nights over the monitoring period. Despite the shorter recording time (including a data gap due to a technical fault in June 2023), this cave recorded the most calls during the monitoring period, including from times that indicate diurnal roosting. Based on the recording data and call patterns it was estimated that up to three individuals at a time were roosting in CWER-16 for a total of 100 days (Appendix K). There was continual presence of bats between 3rd February and 4th March, intermittent roosting by one individual from mid-March to the end of April and estimates of one to two individuals roosting between 5th May and 8th June (Appendix K). For the remainder of the monitoring period (3rd July to end of September), intermittent roosting was observed by one individual for periods of up to seven nights.

There were relatively few calls made from CWER-03 across the 367 nights of recording (data collected 7th September 2022 to 9th September 2023). Calls were made on eight dates in November 2022, and April, August and September 2023 (Appendix K). In April 2023, the timing of the call indicates that an individual was using the cave as a daytime roost (Appendix K).

At CWER-01 (367 nights of recording data collected 7th September 2022 to 9th September 2023), calls were recorded on 25 dates, primarily between June and August 2023 (Appendix K). Day roosting by an individual was recorded on one day in June and three days in August 2023 (Appendix K).

The two caves at Ninga had the second highest number of calls during the 2023 monitoring period (data collected 6th September 2022 to 11th September 2023). At CNIN-03 (370 nights of recording), calls were recorded on 46 nights, with 32 of these indicating day roosting (Appendix K). Most of the day roosting was recorded between May and August 2023, with small number of nights in September 2022. Up to three individuals were recorded for one day at the end of May 2023, and for nine nights in June 2023. At CNIN-09 (370 nights of recording), calls were recorded on 39 nights. Twenty-seven of the dates (27) indicate diurnal roosting by one individual, including for 14 consecutive days in January 2023 and six days in May 2023 (Appendix K).

At CJIM-05 (369 nights of recording, data collected 6th September 2022 to 10th September 2023), calls were recorded on five nights between June and July 2023. On three of these nights, up to two individuals were recorded.

Calls of Pilbara leaf-nosed bats (*Rhinonictis aurantia* 'Pilbara form'; listed as Vulnerable under the EPBC Act and the BC Act) were recorded at four of the eight roosts (CCAT-06, CNIN-03, CNIN-09 and CWER-01) (Appendix K). The timing of calls indicates that there was no daytime roosting in any of these caves during the monitoring period (Appendix K).

3.7 Camera Monitoring

Ghost bats were recorded on four nights in CWER-01: 14th May and 25th, 26th and 27th June 2023. On 14th May and 25th June, individuals were recorded between 0318 and 0338, approximately 3 hours before dawn (0603-0618 hrs, respectively). On 26th and 27th June, they were recorded between 2032 and 2339, approximately 3-6 hours after dusk. A ghost bat was recorded three times on 27th June (Appendix I).

Ghost bats were recorded on two nights in CWER-03: 12th April and 29th July 2023. Two records were made on each night of an individual bat. All were recorded between 0322 and 0444 hours, 1.5 to 2 hours before dawn.

An individual ghost bat was recorded on a single night (15th May 2023) between 0305 and 0307 hours.

Within CJIM-05, ghost bats were all recorded in June 2023, on 26th, 29th and 30th. One record was made on 26th, and two on 29th (all between 0204 and 0301) between approximately 4 and 3 hours before dawn. There were eight recorded sightings of a ghost bat on 30th June between 0000 and 0329, just under 3 hours before dawn

Ghost bats were recorded in two caves at Ninga. At CNIN-03 they were recorded on eight nights: 16th and 20th April 16th, 20th, 21st, 25th and 29th May, and 20th July. All records were made between 0135 and 0446 and were between approximately 4.5 hours and 16 minutes before dawn. The most records of an individual were on the 20th May (four between 0140 and 0402) and 20th July (three between 0153 and 0256). The remaining records were all from one individual sighting. In CNIN-09, ghost bats were recorded on six nights on 19th April, 14th, 24th and 29th May, and 1st and 2nd of July. All were recorded between 0045 and 0521, between approximately 6.5 hours and just under 1 hour before dawn. On 2nd July, there were five sightings between 0045 and 0500. This was the only cave where more than one ghost bat was observed. Two bats were observed as a flyby on 29th May at 0521.

None of the recordings showed roosting for extended periods.

3.8 Microclimate Analysis

The mean temperature within the monitoring caves ranged from 25.51°C (at CWER-14) to 31.4°C (at CJIM-04) (Appendix J). The temperatures were most stable at CWER-09 (Category 3 (C3) cave) with a difference of only 2.54°C. CNIN-03 (C2) had a smaller difference; however, was based on an incomplete dataset. The temperatures were least stable at CJIM-05 (C4) with a 20.64°C difference (Appendix J; Table 3.7).

Table 3.7: Summary of microclimate data from caves where ghost bats were recorded

Area	Cave ID	Temperature		Relative Humidity	
		Mean ($\pm$ SE)	Range	Mean ($\pm$ SE) (%)	Range
Western Ridge	CWER-01	27.81 $\pm$ 0.04	24.07 - 33.11	31.39 $\pm$ 0.13	12.86 - 52.89
	CWER-03	29.56 $\pm$ 0.03	26.75 - 33.98	27.63 $\pm$ 0.12	10.75 - 45.80
	CWER-16	-	-	-	--
	CWER-17	29.35 $\pm$ 0.05	25.49 - 33.77	40.76 $\pm$ 0.13	17.63 - 48.17
Jimblebar/ Ninga	CJIM-05*	27.69 $\pm$ 0.08	16.92 - 37.56	27.06 $\pm$ 0.18	8.77 - 62.73
	CNIN-01	29.4 $\pm$ 0.04	26.00 - 32.45	34.08 $\pm$ 0.19	16.16 - 60.64
	CNIN-03*	28.28 $\pm$ 0.01	27.62 - 29.43	31.33 $\pm$ 0.12	20.86 - 50.00
	CNIN-09	-	-	-	-
	CNIN-13	27.27 $\pm$ 0.07	20.43 - 34.30	38.59 $\pm$ 0.16	13.48 - 60.38

* Data not available for the full monitoring period.

CJIM-07 had the least percentage of recordings within the preferred temperature range of 28-32°C (12.2%), while CCAT-06 had the highest percentage of recordings within the preferred range (100%) (Appendix J).

Category 4 caves tended to have fewer temperature recordings within the preferred range. For example, 13 of the 20 Category 4 caves subject to microclimate monitoring had temperature recordings within the preferred range for less than 30% of the time. In contrast, only two of the 13 Category 3 caves (CNIN-13 and CJIM-09) and none of the Category 2 caves were in the preferred range for less than 30% of the monitoring period. CNIN-01, a Category 3 cave, was one of the roosts with the greatest percentage of temperature recordings within the preferred range for ghost bats (69.67%).

For the caves with temperatures outside of the preferred temperature range, temperatures were generally above the preferred over the summer period and below the preferred during the cooler months (Appendix J).

Relative Humidity

Ghost bats may select roost sites based on humidity; however, the range of humidity levels recorded at sites used by the species varies widely (e.g. from 14% up to 84%) so temperature is considered more important than humidity when assessing roost suitability (Armstrong & Anstee, 2000). The mean RH recorded at the monitored roosts ranged from 24.26% (at CCAT-17) to 61.77% (at CJIM-09) (Table 3.7). The variation in RH was least at CWER-17 with a 30.53% difference between minimum and maximum RH (CNIN-03 had a smaller difference, however the available data was not representative of the entire monitoring period, as was the case for CJIM-09). The variation in RH was greatest at CWER-14 (56.92% difference between minimum and maximum RH) (Table 3.7).

3.9 Habitat and Disturbance Monitoring

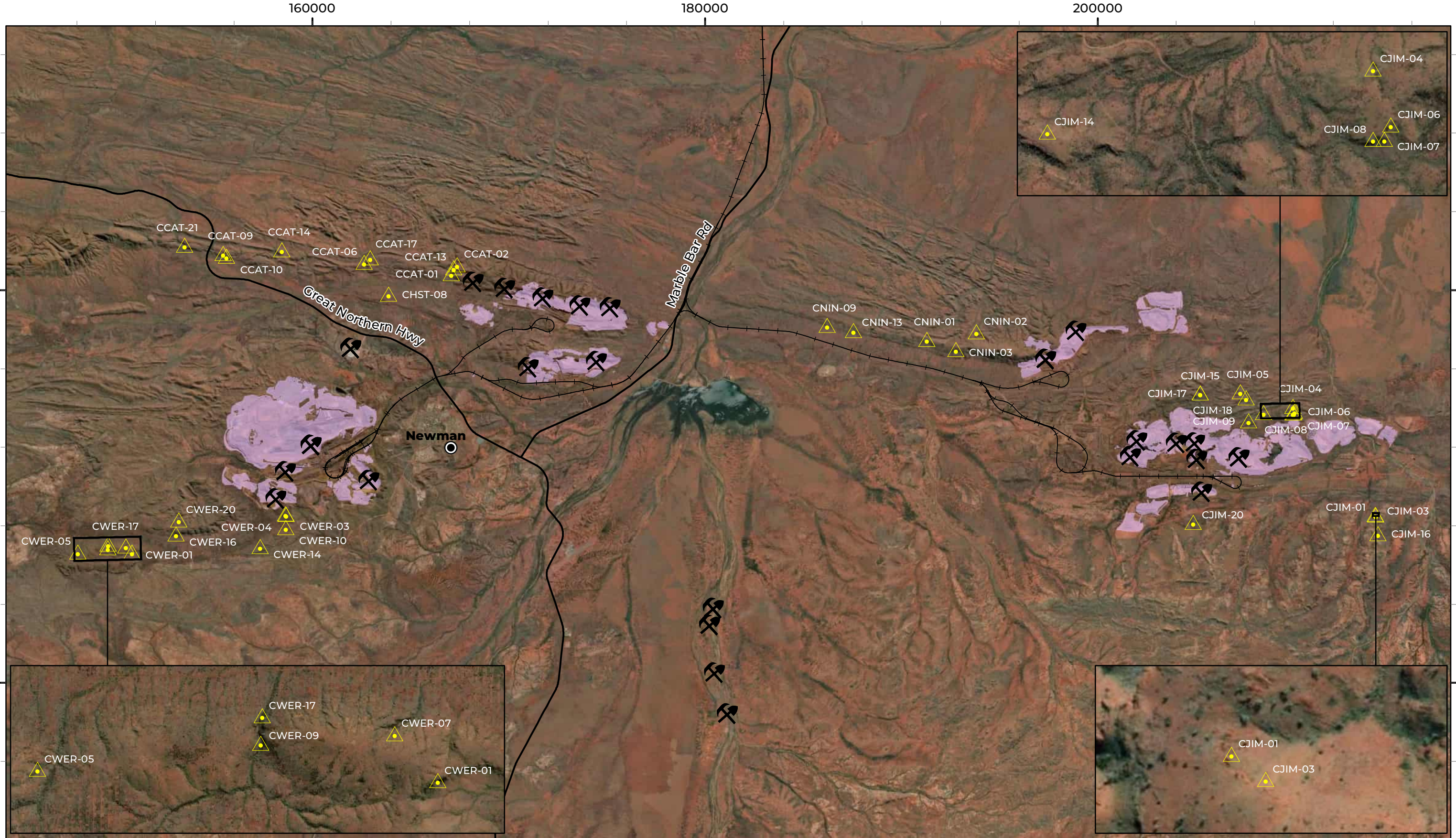
Photo point monitoring suggested all caves were in excellent condition and there were no obvious signs of disturbance (including fire) at the entrance to, or immediately in front of, these caves during the monitoring period.

As of 30th June 2023, approximately 7,293.64 ha of land associated with active mining was cleared in the Study Area (area calculated using a BHP WAIO supplied layer) (Figure 3.2). This is an increase of 145.64 ha in clearing compared to 2022 (7,148 ha cleared). Construction had not yet commenced at Western Ridge. The closest mining activity is associated with Orebody 35 and 24 and Jimblebar, but mining activity is also present at Whaleback and surrounding orebodies.

Most monitoring caves were marginally closer to active mining related disturbance (10-20 m) in 2023 compared to 2022 (Table 3.8; Figure 3.2; Appendix L). Ten caves, including CWER-03 where ghost bats were recorded, are less than 1 km from mining related disturbance, with caves CJIM-06, CJIM-07, CJIM-08 and CWER-04 less than 500 m to disturbance (Table 3.8; Figure 3.2; Appendix L).

Table 3.8: Distance from disturbance of caves where ghost bats were recorded

Area	Cave ID	Category	Distance (km) 2023	Distance (km) 2022	Difference Distance 2022 vs 2023 (km)
Western Ridge	CWER-01	2	5.83	5.81	-0.02
	CWER-03	2	0.52	0.53	0.01
	CWER-16	3	3.53	3.55	0.02
	CWER-17	3	6.62	6.64	0.02
Jimblebar/ Ninga	CJIM-05	4	1.50	1.50	0
	CNIN-01	4	4.78	4.78	0
	CNIN-03	2	3.23	3.24	0.01
	CNIN-09	3	8.02	8.02	0
	CNIN-13	3	8.53	8.51	0.02



LEGEND

Monitoring Cave	State Road	Mining Disturbance (30/06/2023)
Operating Mine	Rail	

Scale 1:184,000

0 3 6 Km

Coordinate System: GDA 1994 MGA Zone 51
Projection: Transverse Mercator
Datum: GDA 1994 Created 20/11/2024

BHP WAIO
Eastern Pilbara
Ghost Bat Monitoring
2022-2023

Figure 3.2: Mining related disturbance

4 Discussion

Ghost bat presence within caves has been determined using four different monitoring techniques: direct observation, scat observations, ultrasonic recordings, and motion cameras. How ghost bats use the caves is determined from the scat monitoring, hormone analysis, genetic analysis and motion cameras. Finally, to provide insight into how they use their environment, diet analysis, microclimate monitoring, and when successful, GPS tracking has been used.

Ghost bat evidence has been recorded in 21 of the 41 monitoring caves, with evidence recorded in 19 caves since September 2021 (the start of the monitoring program) (Table 4.1). The remaining 20 monitoring caves have never recorded ghost bat evidence to date. For two (CCAT-02 and CCAT-09) of the 41 monitoring caves, there has been no ghost bat evidence recorded since the monitoring program commenced (Table 4.1). There are only seven caves (CWER-01, CWER-03, CWER-17, CJIM-05, CNIN-01, CNIN-03 and CNIN-09) that have shown evidence of ghost bat presence over the two years of monitoring (Table 4.1).

Scat monitoring continues to provide a non-invasive technique to determine cave usage within known monitoring periods and, where DNA and hormone analysis can successfully be undertaken, the ability to determine individual presence and movement. Scat numbers fluctuate significantly (Table 4.2). For example, CWER-03 has been visited on seven occasions since the commencement of monitoring. Scats have been present on sheets on four of these occasions, with numbers varying from 200 in February 2022, June 2022 and January 2023, and only one in June 2023 (Table 4.2). In the one visit between the June and January collections (September 2022), there were no scats present (Table 4.2). In the three visits since January 2023, there has only been one scat present (Table 4.2). This cave is currently located 0.52 km from disturbance (land clearing), which has become closer by 0.01 km since June 2022 (0.53 km).

The cave with the highest number of scats on sheets in 2023 was CNIN-01 (380 total, 230 in June 2023 and 150 in September 2023), yet this had none during the four visits in 2022 (Table 4.2). Scats collected from this cave in June/July had elevated progesterone, indicating the presence of at least one pregnant female (discussed later in this section). In 2022, the cave with the highest number of scats was CWER-03. It is logical to assume that caves with high numbers of scats are more important to ghost bats, if the sampling period is long enough to determine trends. Based on this, the caves that have had the highest deposition during the monitoring period (limited to the sheets where time frames can be determined), were CWER-03 and CNIN-01 in 2023 and CWER-03 in 2022 (Table 4.2). The highest use during any one period was at CNIN-01, with an average scat deposition rate of 1.83 scats/day between April and June 2023 (Table 4.2).

This pattern of fluctuating cave use (determined via scat deposition) is consistent with other regions of the Pilbara (especially the area around South Flank/Mining Area C where most work to date has been done) and may be influenced by a number of factors including, the availability of food resources and possible changes to cave temperatures.

Table 4.1: Summary of ghost bat presence

Area	Cave ID	Category	Ghost Bat 2023	Ghost Bat 2022	Ghost Bat Pre-2022
Western Ridge (11*)	CWER-01	2	S(P), U, C	S, U	S*
	CWER-03	2	S(P), U, C	O, S(P), U	
	CWER-16	3	U		
	CWER-17	3	S	S	
	CWER-20	4		S*	
Jimblebar/ Ninga (20*)	CJIM-03	2			O, S*
	CJIM-05	4	U, C	S(P)	
	CJIM-09	3			S*
	CJIM-15	4		S	
	CJIM-16	3		S(P)*	
	CJIM-19	4			O
	CJIM-20	4		S	
	CNIN-01	3	S, S(P)*	S*	U
	CNIN-03	2	S(P), U, C	U, S	U, S*
	CNIN-09	3	U, C	O, U, C	U
Cathedral Gorge (10*)	CNIN-13	3	S, O		S*
	CCAT-01	3			S*
	CCAT-02	3			S*
	CCAT-06	3		U	
	CCAT-09	3			S*
	CCAT-21	3			U, S*

O = observation, S = scat on sheet, S* = scat incidentally observed (not on sheets), S (P) = elevated or intermediate progesterone levels in scat, U = ultrasonic record, and C = camera record. * = number of caves monitored in the area

Table 4.2: Scat deposition and rate from 2021 to 2023 monitoring

Area	Cave ID	Trip 1 Sept- 21	Trip 2 Sept- 21	Trip 3 Feb- 22	Trip 4 Jun- 22	Trip 5 Sep- 22	Trip 6 Jan- 23	Trip 7 Apr- 23	Trip 8 Jun- 23	Trip 9 Sep- 23
Western Ridge	CWER-01	-	-	2 (-)	0	0	0	0	8 (0.34)	0
	CWER-03	-	-	200 (0.38)	200 (0.8)	0	200 (0.92)	0	1 (0.12)	0
	CWER-17	-	-	-	0	2 (0.15)	0	0	8 (0.34)	15 (0.46)
	CWER-20	-	-	3 (-)	0	0	-	-	0	0
Jimblebar/ Ninga	CJIM-03	1,000 (-)	0	0	0	0	0	0	0	0
	CJIM-05	-	0	0	35 (0.57)	0	0	0	0	0
	CJIM-09	-	15 (-)	0	0	0	-	0	0	0
	CJIM-15	-	0	0	2 (0.10)	0	0	0	0	0
	CJIM-16	-	-	-	-	40 (-)	0	0	0	0
	CJIM-20	-	-	0	1 (0.01)	0	-	-	0	0
	CNIN-01	-	0	0	0	2 (-)	0	0	230 (1.83)	150 (1.45)
	CNIN-03	-	2,000 (-)	0	5 (0.22)	500 (-)	2 (0.12)	0	0	10 (0.38)
	CNIN-13	-	-	5 (-)	0	0	0	0	12 (0.41)	50 (0.84)
Cathedral Gorge	CCAT-01	-	5 (-)	0	0	0	-	0	0	0
	CCAT-21	-	5 (-)	0	0	0	-	0	0	-

During the 2023 monitoring, evidence of pregnant females (determined via scats with elevated progesterone levels) was found in four caves; two at Western Ridge (CWER-01 and CWER-03) and two at Ninga (CNIN-01 and CNIN-03) (Table 4.1). Only one of these caves also had pregnant females in 2022 (CWER-03). Two caves at Jimblebar had pregnant females in 2022 (CJIM-05 and CJIM-06) and none had pregnant females in 2023.

During both the 2022 and 2023 monitoring, scats with elevated progesterone have been recorded in the mid-year collection samples in both 2022; two caves, CWER-03 and CJIM-05, collected between February and June, and three caves, CWER-01, CNIN-01 and CNIN-13 collected between April and June/July. The current literature states that within the Pilbara, mating typically occurs in July and August, with the females giving birth approximately three months later, in late October or early November (Richards *et al.*, 2008). Armstrong and Anstee (2000) observed a pregnant female in early September 1998 and females with juveniles attached in November and December 1998. Juveniles have also been observed in November and December at Lalla Rookh and Mining Area C (Biologic, 2016).

More widely, ghost bat reproduction is variable across their range, suggesting that there are differences in the timing of mating and birth between the Pilbara and other areas. At Pine Creek in the Northern Territory, observations of juveniles and unfurred suckling young, suggested the birth of the offspring in August or September (Hoyle *et al.*, 2001; Schulz & Menkhorst, 1986). Three females caught from Pine Creek in June 1984 were pregnant with embryos at a stage consistent with conception around the second week of May (assuming an estimated gestation of 11–12 weeks) and births predicted to typically occur in early August (Pettigrew *et al.*, 1986). There is variability of births recorded in captivity, which although usually reported as between September and November, have been observed in January, April and July (Jones, 2008).

The presence of progesterone in scats deposited between February and June suggests the presence of at least one pregnant female. Progesterone levels do increase and decline during early and late pregnancy, and also degrade over a period of time after deposition (Keeley *et al.*, 2023). High levels (i.e. greater than 1,000ng/g) were present in 11 samples from 2023 and there were no scats present on the sheets during the previous two collection trips (where deposition would have occurred over a period of nine months).

Reproductive strategies in bats are diverse and may include postpartum oestrus, sperm storage, delayed implantation, or embryonic diapause, all of which are largely controlled by hormones (Crichton & Kruttsch, 2000). The variability in timing of birth in wild and captive populations of ghost bat suggests that it has some level of flexibility or control in the timing of reproduction due to reproductive strategies, which would possibly favour the best timing for abundant food resources for newly weaned young (Keeley *et al.*, 2023). In the case of the Jimblebar bats, it is considered highly likely that bats can be pregnant during the middle of the year (between February and June/ July), with either births occurring during the middle of the year, with longer pregnancies than typically reported (considered most likely), or the birthing of pups outside the period currently reported that has not yet been detected.

For this monitoring period, the levels of elevated progesterone, alongside the genetic, ultrasonic and camera data, were not pronounced enough to suggest that caves were being used as maternity diurnal roosts for the 2022 birthing period or the late pregnancy period in 2023. But rather the results of short temporary visitations by a small number of pregnant individuals, leading to the question of where the bats inhabiting the Study Area are actually breeding and residing.

Thirteen individuals (four females, eight males and one undetermined) have been detected from the Study Area to date, with all but one recorded during the two years of monitoring (samples from 2019 added an additional individual which to date has only been recorded from that year). There is evidence of long-term presence at Western Ridge for three ghost bats. The female bat, #133 was first identified in 2016 from CWER-03, and it has been recorded almost every year of monitoring either in this cave or CWER-01, approximately 8 km to the west. To Biologic's knowledge, the record of this bat again in 2023 makes it the longest period of detection for an individual ghost bat in the wild. This individual is also the only one detected outside the Study Area, when it was recorded from a cave at Punda, approximately 45 km to the north in 2017. Other than this large movement, most of the repeat samples have been from the same cave during the same sampling period, or from caves less than 15 km apart.

Individual bats have been identified from four caves at Western Ridge (CWER-01, CWER-03, CWER-16 and CWER-17) and three at Ninga (CNIN-01, CNIN-03, and CNIN-13). Genetic analysis has only identified bats at Ninga since the monitoring commenced in 2023, and all to date are males. Nine individuals have been detected at Western Ridge, with three identified from scats prior to the monitoring period. The genetic analysis has not yet identified any individual genotypes at Jimblebar or Cathedral Gorge.

Scat numbers fluctuate greatly between collection dates for a cave; yet low numbers of scats have not limited the ability to detect multiple individuals at a cave. For example, CWER-01 had eight scats collected in June 2023, and three individuals were identified from these (one female and two males). Whilst CWER-01 has had multiple individuals detected in it over multiple years (including pregnant females), the low scat numbers suggest that, for the monitoring period at least, it is being used as a feeding cave, or else the main roost is not accessible for scat monitoring.

Of the individuals that have been identified by genotypes, the four males recorded across the 2023 sampling period had the highest number of scats collected per individual: 873 (CWER-03, January and June, 33 scats); 906 (CWER-17, June and September, 19 scats); 907 (CNIN-13, July and September, 31 scats); and 908 (CNIN-01, April, July and September, 114 scats). In comparison, the female #133 has been identified from caves CWER-01 in 2023 and CWER-03 in 2019 from only four scats (Table 4.3).

Table 4.3: Cave use by genotyped individuals and scat deposition

Area	Cave ID	Trip 1 Sept-21	Trip 2 Sept-21	Trip 3 Feb-22	Trip 4 Jun-22	Trip 5 Sep-22	Trip 6 Jan-23	Trip 7 Apr-23	Trip 8 Jun-23	Trip 9 Sep-23
Western Ridge	CWER-01	-	-	2 (-)	0	0	0	0	8 (0.34)	0
									#133F (2) #508M (2) #906M (1)	
	CWER-03	-	-	200 (0.38)	200 (0.8)	0	200 (0.92)	0	1 (0.12)	0
				#877F (1)			#856F (11) #873M (33) #874M (2)	#135F (9)	#133F (2)	
	CWER-17	-	-	-	0	2 (0.15)	0	0	8 (0.34)	15 (0.46)
									#906M (7)	#906M (11)
Jimblebar	CNIN-01	-	0	0	0	2 (-)	0	0	230 (1.83)	150 (1.45)
								#908M (5)	#908M (39)	#908M (69)
	CNIN-03	-	2,000 (-)	0	5 (0.22)	500 (-)	2 (0.12)	0	0	10 (0.38)
										#875M (7)
	CNIN-13	-	-	5 (-)	0	0	0	0	12 (0.41)	50 (0.84)
									#907M (12)	#907M (19)

Note: scat deposition rates are included in parenthesis "()". **Bold** = The highest deposition rate per cave over the monitoring assessment

Relatedness was observed in seven of the 13 individuals. Only two (both females) have a full-sibling or parent-offspring relationship: (#856 and 877), which have a second-degree relationship with #135 (which then has a second-degree relationship with #133). These five individuals are therefore related and have all been recorded in CWER-03. The second group of related individuals (all second degree) are all males (#508, 906 and 908). Numbers 508 and 906 have both been recorded at Western Ridge (CWER-01, -16 and -17), whilst #908 has been recorded at Ninga (CNIN-01). Given the relatively short period of monitoring and the reasonably small dataset of 13 individuals, it is anticipated that more insight into the relatedness and (family) clades will be obtained as the monitoring progresses.

Published accounts indicate that the ghost bat diet comprises bats, other small mammals, birds, frogs and geckos (Claramunt *et al.*, 2018). Toop (1985), based on studies at Mount Etna in Central Queensland, suggested that their diet changed seasonally, with insects being the main food source during the warmer months and birds and mammals are primarily consumed in the cooler months. The dietary analysis indicates that the main food source for the ghost bats prior to the period of collection at CNIN-01 in September 2023 were primarily budgerigars, other small unidentified birds and house mice. Budgerigars and house mice are primarily granivorous and are therefore highly reliant on the presence of seeding shrubs and grasses, which is dictated largely by rainfall. Further studies on the presence of prey species, and what those prey species consume, could help predict foraging areas and therefore feeding or day roosts for ghost bats and is a recommendation for future study.

The two caves where the highest number of scats have been recorded (from one collection period) are CWER-03 and CNIN-01.

As of the 30th June 2023, approximately 7,293.64 ha of land associated with active mining had been cleared in the Study Area, which is 145.64 ha more compared to 2022 (7,148 ha cleared). Ten monitoring caves (including CWER-03 where ghost bats were recorded) were located within 1 km of mining disturbance (i.e. active mining) and the nearest caves (CJIM-06, CJIM-07, CJIM-08 and CWER-04) were within 500 m of mining disturbance. Only minor changes in the distance of caves to mining disturbance were recorded in 2023 (mostly changes of 10-20 m). Given the sporadic and low visitations of ghost bats in the area, it is difficult to determine any trends related to distance to disturbance. Further monitoring should assist with determining this.

Caves and roosts used by ghost bats can be classified into four categories defined by (Cramer *et al.*, 2022) based on type and frequency of use. There are currently no known Category 1 roost sites within the Study Area, or Hamersley subregion generally. To date, there are only a handful of documented permanent roost sites in the Pilbara, which are largely/ exclusively restricted to abandoned mines and adits in the Chichester subregion.

Two of the Category 3 caves – CNIN-01 and CWER-16 – warrant consideration for changes in status, from Category 3 to Category 2 (non-maternity diurnal roost) caves. CNIN-01 was first assessed in September 2021 at the commencement of monitoring, and on the seventh visit in June 2023 there were 230 scats within it, with a deposition rate of 1.83 scats per day since April 2023. Compared to other Category 2 caves within the Study Area this cave has a higher average temperature, smaller range in temperatures and higher relative humidity (i.e. may be more stable and therefore suitable for roosting than the other caves). Pregnant females were identified in this cave between April and July 2023 - three of 71 scats collected on 2 July had elevated progesterone (range 2,757-3,697 ng/g). A continued presence of ghost bats (including pregnant females) within it over the cool winter months (average minima at Newman during May, June and July 2022 were lower than the long-term average), suggests it may be used as a Category 2 roost (maternal roost). Therefore, CNIN-01 may be considered a maternity/ diurnal roost cave with regular (but not continuous) ghost bat occupancy that is capable of supporting one or more reproducing females and their habitat.

CWER-16 recorded the most calls during the monitoring period, with timing of calls indicating that diurnal roosting was occurring. This is the only technique that has recorded ghost bats over the two years of monitoring, although it is noted that scat collection was not able to be undertaken at this cave due to access (safety) constraints. Based on the high number of calls and the timing that indicates diurnal roosting, this cave should be reclassified as Category 2 (diurnal roost).

In addition, CJIM-19 (Category 4 cave) warrants consideration to downgrade the status from a Category 4 to a Category 5 cave based on its characteristics, lack of suitability for roosting ghost bats and lack of recent evidence of ghost bats. Further assessment of CJIM-19 will be conducted in 2025.

Daytime roosting was recorded via the ultrasonic at CNIN-03 and CNIN-09, with roosting occurring over consecutive days at both caves. Hormone analysis of scats at CNIN-03 has also confirmed its use by at least one pregnant female, though in low numbers. Intermittent daytime roosting has also been indicated by calls at CWER-01 and CWER-03 which suggests sporadic visits from ghost bats. Hormone analysis of scats at CWER-03 has also confirmed its use by pregnant female/s.

The mean temperature recorded at the monitored roosts ranged from 25.51°C (at CWER-14) to 31.4°C (at CJIM-04). The temperatures were most stable at CWER-09 during 2022 and 2023, and least stable at CJIM-05. CJIM-07 had the least percentage of recordings within the preferred temperature range of 28-32°C (12.2% of recordings) while CCAT-06 had the highest percentage of recordings within the preferred range (100%). As expected, Category 4 caves were less likely to have temperature recordings within the preferred range. Ghost bats were recorded using caves which were subject to a wide range of temperatures. For example,

ghost bats used caves with mean temperatures ranging from 27.27°C (CNIN-13, a Category 3 roost) to 29.56°C (CWER-03, a Category 2 roost). There were numerous caves that had ideal temperatures for ghost bats (e.g. mean within the preferred, highly stable and higher percentage of recordings within the preferred range); however, no evidence of ghost bat presence was recorded during the monitoring period at these caves (e.g. CJIM-14, CWER-09, CCAT-06, CCAT-09).

Although individuals in the area currently appear to be stable, due to the inherently low numbers and sparse distribution, ongoing monitoring is recommended to enable the future stability of the local population to be monitored, particularly in the context of nearby mining operations.

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Appendix A: Species Profile

The ghost bat occurs in disjunct colonies across northern Australia (TSSC, 2016). The distribution of the ghost bat is restricted to geographically discontinuous populations across northern Australia; these populations are highly structured, and genetically distinct at both regional and local scales (Worthington-Wilmer *et al.*, 1994). In Western Australia, the Pilbara population is located at the southern limit of the species' range and is geographically isolated and separated from the Kimberley population (Bat Call, 2021) by a distance of approximately 600 km. The Pilbara population is estimated to comprise approximately 1,850 individuals (Bat Call, 2021).

In the Pilbara, mating typically occurs in July and August, with the females giving birth approximately three months later, in late October or early November (Cramer *et al.*, 2022; Richards *et al.*, 2008).

Roost sites include caves, rock crevices and disused mine adits (Bat Call, 2021) with stable temperatures of around 28°C (Baudinette *et al.*, 2000). While ghost bats use caves with varying levels of relative humidity, humid caves are used for rearing young (Armstrong & Anstee, 2000). The largest colonies known to occur in the Pilbara are in disused mines (e.g., 500+ individuals at Lalla Rookh), while natural caves typically support up to ten individuals (Bat Call, 2021; Cramer *et al.*, 2022; TSSC, 2016). Natural roosts generally comprise deep, complex caves beneath bluffs or low rounded hills composed of Marra Mamba or Brockman Iron Formation (Armstrong & Anstee, 2000) and Robe Pisolite channel iron deposit geology (Bat Call, 2021).

Caves and roosts used by ghost bats can be classified into four categories (Bat Call, 2021) as follows:

- **Category 1 maternity/ diurnal roost sites with permanent ghost bat occupancy:** Maternity/ diurnal roost caves with permanent ghost bat occupancy. There are several documented permanent roost caves and underground mines in northern Australia. These may be abandoned underground mines;
- **Category 2 maternity/ diurnal roost caves with regular occupancy:** Maternity/ diurnal roost caves with regular (but not continuous) ghost bat occupancy that is capable of supporting one or more reproducing females and their habitat. These may be abandoned underground mines;
- **Category 3 diurnal roost caves with occasional occupancy:** There are many caves and adits where one to a few ghost bats roost occasionally, or rarely; and
- **Category 4 nocturnal roost caves with opportunistic usage:** shallow caves, shelters and deep overhangs that support opportunistic usage for resting and feeding.

In addition to the above categories, a further category - Category 5 - was defined to categorise caves that were not suitable for use by the ghost bat (e.g. due to a restricted entrance or the dimensions of the cave). Category 5 is not described by Bat Call (2021), but is used here for consistency with the terminology used in this guide.

Ghost bats prefer to forage on productive plain areas with thin mature woodland over patchy or clumped tussock or hummock grass on sand or stony ground (Bat Call, 2021). Ghost bats typically emerge from their roosts at dusk and commence hunting 1 to 1.5 hours after sunset, initially for a period of about two hours and then again just prior to sunrise; intermittent hunting also occurs through the night (Boles, 1999). Ghost bats have a 'sit and inspect' foraging strategy where they hang from a perch and, once their prey is detected, they drop on it or capture it in the air or glean it from the ground/ vegetation (Boles, 1999). Preferred perches include isolated trees and trees on the edge of thin thickets on plains, or trees along the edges of watercourse woodlands (Bat Call, 2021). Ghost bats are known to consume large insects, frogs, lizards, birds, small mammals and other bats (Boles, 1999; Richards *et al.*, 2008; Start *et al.*, 2019). Ghost bat diet in the Pilbara consists mainly of small mammal and bird species, and to a lesser extent, reptiles (geckoes and skinks) and amphibians (Claramunt *et al.*, 2018).

Ghost bats move between caves seasonally, or as dictated by weather conditions, so require a range of cave sites (Hutson *et al.*, 2001). Outside of the breeding season, male bats are known to disperse widely (100+ kilometres), especially during the wet season (Ottewell *et al.*, 2017; Worthington-Wilmer *et al.*, 1994). Females are more likely to stay close to, or return for breeding to, the maternity roosts from which they originated (Worthington-Wilmer *et al.*, 1994). Ghost bats forage over areas up to 12 km from their diurnal roost (Augusteyn *et al.*, 2018). Bats transiting to distant sites have also been recorded between 20 and 30 km from their diurnal roost in a night (Augusteyn *et al.*, 2018; Bat Call, 2021).

The most severe threats to the species is the destruction and disturbance of habitat due to mining operations and poisoning by cane toads (TSSC, 2016). Disturbance to their roosts from other infrastructure developments and changes to and loss of foraging habitat also pose significant threats (Cramer *et al.*, 2022). Presently, there is no recovery plan for the species. The Conservation Advice for the ghost bat identifies the establishment (or enhancement) of monitoring of populations at key sites and where impacts from mining are occurring or likely as a high priority (TSSC, 2016).

Appendix B: Standard Monitoring Methods

C.1 Selection of Monitoring Sites

Prior to the commencement of monitoring in 2021, caves are selected based on their potential value to ghost bats (Category 1 to 4 below) and to meet compliance or research requirements for the relevant area. Reference caves outside the area of interest are included where possible to enable regional comparisons.

C.2 Ghost Bat Observations

Each cave visitation is undertaken by at least two personnel: 1) an 'inspector' will enter the cave, and a 'spotter' will wait at the entrance of the cave to ensure the safety of the inspector and record any accidentally flushed bats. A cave entrance assessment is completed prior to entry to determine whether each cave is safe to enter. For ethical reasons, the inspector will enter the cave with caution to avoid flushing any ghost bats roosting inside the cave. If the inspector observes roosting ghost bats in a chamber, they will withdraw from the chamber and continued monitoring other areas of the cave (if able to do so whilst leaving the ghost bats undisturbed). An exception to this is when trapping is undertaken to obtain ghost bats to be used for GPS tracking. The ghost bat is distinctive from all other cave-dwelling bats within the Pilbara region, being both larger in size and lighter in colouration, so is easily recognised if observed. Any signs of reproduction (presence of pregnant females or pups) are noted.

C.3 Scat Counts and Collection

Generally, during the first visit at each cave, black sheets are deployed below sections of cave ceiling which are considered likely to support roosting by ghost bats or where scats have previously been deposited. The number of sheets within each cave may vary according to the number and spatial spread of scat piles. Provided no ghost bats are roosting inside the cave; the inspector will search the sheets in each cave for scat material. Note, scats and middens are distinctive for this species, with scats being more than twice the size of other cave-dwelling species.

The number of scats occurring on-sheet(s) are counted and recorded prior to any collection. Total scats recorded on-sheet(s) are later used to determine a scat deposition rate (the number of scats recorded divided by the number of days since sheets were last cleared). To compare scat deposition graphically, a square root transformation is applied.

C.4 Hormone Analysis

Faecal samples are analysed for progesterone metabolite concentrations by enzyme-immunoassay (EIA). Prior to analysis for hormone concentrations, each faecal sample is extracted using a basic hormone extraction procedure (Keeley et al., 2012a; Palme et al., 2013). Faecal samples are subsampled to a weight of either 0.1 ± 0.02 or 0.05 ± 0.002 grams

(g) to which 5 ml of 80% methanol was added. Samples are rotated gently overnight, centrifuged at 1000 g for 10 minutes and then decanted and stored at -20°C until analysis. The supernatant is diluted 1:20 to 1:1000 (dependant on concentration) in assay buffer prior to analysis. Faecal progesterone metabolite concentrations are quantified by double antibody EIA using a goat anti-mouse IgG (Arbor Assays, USA), monoclonal progesterone antiserum (CL425), horseradish peroxidase conjugated label (both provided by C. Munro, University of California-Davis, Davis, USA) and progesterone (Sigma Aldrich Australia Ltd.) standards as previously described with minor modifications (Keeley et al., 2012b).

The antiserum (1:80,000) is incubated on a microtitre plate overnight, horseradish peroxidase conjugate (1:400,000), standards (0.016 - 4 ng/ml) and samples are loaded (50 µl/well) onto the plate and the EIA was performed as described (Keeley et al., 2012b; Pollock et al., 2010). Intra and inter-assay coefficients of variation are both <10%. Cross-reactivities for the EIA antibodies were as described (Graham et al., 2001). Hormone concentrations are expressed as nanograms of hormone metabolites per gram of faeces (ng/g). Progesterone levels are considered to be of an intermediate level for results greater than 700ng/g but less than 999ng/g. Progesterone levels are considered to be elevated for results of 1,000ng/g or greater. Intermediate and elevated levels of progesterone indicate pregnancy of an individual.

These results aid in determining if breeding is occurring within the Study Area. In combination with results of successful genetic analysis of scats, the occurrence and abundance of specific pregnant individuals at each cave are utilised to identify the presence of potential maternity roosts.

C.5 Genetic Analysis

In order to undertake genetic analysis, firstly DNA extractions are taken from the scats by Biologic's in-house genetics laboratory.

Samples collected are genotyped at 49 SNPs using a new high-throughput SNP genotyping method (Thavornkanlapachai *et al.*, 2024). Samples are filtered from the SNP genotyping dataset to keep samples with an amplification success rate of ≥77% and loci with an amplification rate ≥80% and set up a mismatch threshold (*h*) of five.

SNP genotypes for scat samples were processed using a custom R package 'ScatMatch' (Huntley, 2021). The analysis groups scats are based on genotype similarity, i.e. by number of allelic mismatches, to identify the number of unique genotypes present in the sample. All unique genotypes identified in the study are matched to DBCA's genotype database to determine the number of 'recaptured' individuals. Molecular sexing of unique individuals is carried out using four custom-designed ghost bat sex-linked markers (DDX3Y, SRY, Zfy and Zfx) as described in (Ottewell *et al.*, 2020).

Population genetic diversity is calculated based on the total number of individuals detected in this study. Calculations are based on the loci shared by all individuals after filtering.

Summary population genetic diversity statistics, including observed (H_o) and expected heterozygosity (H_e), number of alleles (N_a) and the inbreeding coefficient (F_{is}) are calculated in GENALEX v6.5 (Peakall & Smouse, 2012). GENALEX is also used to estimate the mean relatedness (r) and pairwise genetic relatedness. The program NeEstimator is used to estimate the contemporary effective population size (N_e) using the Linkage Disequilibrium method.

C.6 Ultrasonic Analysis

SongMeter SM4BAT-FS bat detectors (SM4; Wildlife Acoustics, USA) powered by an external solar power supply are used to monitor audio and/or ultrasonic and/or infrasonic calls. Recorders are preconfigured to activate at astronomical sunset each day and deactivate at astronomical sunrise the following morning. Settings are adjusted to record calls for the ghost bat, as well as any other cave dwelling bat species occurring in the region, with a frequency recording range of 12–192 kilohertz (kHz). Data is analysed on all recorded nights at the eight roosts for all species of bats, including ghost bats and Pilbara leaf-nosed bats (*Rhynonictis aurantia*). All recordings are analysed using standardised bat call detection techniques. Raw files are first scanned for ghost bat calls using Kaleidoscope software (Wildlife Acoustics, USA), then reviewed for significant times and call numbers using Cool Edit software (Adobe, USA). A recording night is considered from sunset to sunrise the following day.

C.7 GPS Tracking

The trapping set up involves placing a large cotton sheet across the cave entrance before one person walks into the cave to flush any potential ghost bats into the sheet. The second person is in place to capture the animal when it becomes entrapped using a large cotton bag or towel. A third person remains outside the entrance of the cave for safety reasons and to record any escaped bats. Captured individuals are processed: weighed, sexed, forearm length measured and tagged with a GPS tracking device.

C.8 Camera Traps

Motion sensor cameras are placed below sections of cave ceiling considered most likely to be used by ghost bats or where scats had previously been deposited. The cameras are positioned on the floor looking up or toward the location ghost bats are most likely to hang.

C.9 Habitat and Disturbance Monitoring

Two photo monitoring points, marked with fence droppers, are established at either the cave's entrance or the habitat surrounding the cave. In places where caves are in close proximity, a single landscape photo is taken to include both caves within the context of the surrounding habitat. Repeat monitoring photos enable a pictorial record of habitat changes over time.

Appendix C: Personnel Experience

Appendix Table 1: Personal experience

Personnel	Position and Role	Qualification	Experience
Chris Knuckey	Principal Zoologist/	BSc (Hons) Environmental Management	13 years' EIA (consulting) 13 years' field survey 13 years' vertebrate zoology/ ecology
Belinda O'Connell	Principal Zoologist/ Ecologist	BSc (Hons) Zoology	22 years' EIA (consulting) 22 years' field survey 22 years' vertebrate zoology/ ecology
Hannah Anderson	Senior Zoologist	BSc (Hons) Conservation and Wildlife Biology	6 years' EIA (consulting) 12 years' field survey 12 years' vertebrate zoology/ ecology
Stephen McGrath	Zoologist	BSc Environmental Science and Conservation Biology	8 years' EIA (consulting) 6 years' field survey 2 years' vertebrate zoology/ ecology
Aleesha Turner	Zoologist	BSc (Hons) Zoology and Wildlife Biology	3 years' EIA (consulting) 5 years' field survey 5 years' vertebrate zoology/ ecology
Georgina Mattner	Zoologist	BSc Animal Ecology	2 years' EIA (consulting) 3 years' field survey 2 years' vertebrate zoology/ ecology
Sammy Alatas	Zoologist	NA	1 year EIA (consulting) 1 year field survey 1 year vertebrate zoology/ ecology

Appendix D: Sampling Tables

Appendix Table 2: Sampling caves and survey timing

Area	Cave ID	Cave Category	Trip 6 Jan/Feb 2023	Trip 7 Apr 2023	Trip 8 Jun/Jul 2023	Trip 9 Sep 2023	Total Visitations
Western Ridge	CWER-01	Category 2	X	X	X	X	4
	CWER-03	Category 2	X	X	X	X	4
	CWER-04	Category 4	X	X	X	X	4
	CWER-05	Category 4	-	X	X	X	3
	CWER-07	Category 4	X	X	X	X	4
	CWER-09	Category 4	X	X	X	X	4
	CWER-10	Category 3	X	X	X	X	4
	CWER-14	Category 4	X	X	X	X	4
	CWER-16*	Category 3	X	X	X	X	4
	CWER-17	Category 3	X	X	X	X	4
	CWER-20	Category 4	-	X	X	X	3
Jimblebar/ Ninga	CJIM-01	Category 4	X	X	X	X	4
	CJIM-03	Category 2	X	X	X	X	4
	CJIM-04	Category 4	X	X	X	X	4
	CJIM-05	Category 4	X	X	X	X	4
	CJIM-06	Category 4	X	X	X	X	4
	CJIM-07	Category 4	X	X	X	X	4
	CJIM-08	Category 4	X	X	X	X	4
	CJIM-09	Category 3	-	X	X	X	3
	CJIM-14	Category 3	X	X	X	X	4
	CJIM-15	Category 4	X	X	X	X	4

Area	Cave ID	Cave Category	Trip 6 Jan/Feb 2023	Trip 7 Apr 2023	Trip 8 Jun/Jul 2023	Trip 9 Sep 2023	Total Visitations
	CJIM-16	Category 3	X	X	X	X	4
	CJIM-17	Category 4	X	X	X	X	4
	CJIM-18	Category 4	X	X	X	X	4
	CJIM-20	Category 4	-	-	X	X	2
	CNIN-01	Category 3	X	X	X	X	4
	CNIN-02	Category 3	X	X	X	X	4
	CNIN-03	Category 2	X	X	X	X	4
	CNIN-05	Category 4	X	-	-	-	1
	CNIN-09*	Category 3	X	X	X	X	4
	CNIN-13	Category 3	X	X	X	X	4
Cathedral Gorge	CCAT-01	Category 3	-	X	X	X	3
	CCAT-02	Category 3	-	X	X	X	3
	CCAT-06	Category 3	X	X	X	X	4
	CCAT-09	Category 3	-	X	X	X	3
	CCAT-10	Category 4	-	X	X	-	2
	CCAT-13	Category 3	-	X	X	X	3
	CCAT-14	Category 4	-	X	X	X	3
	CCAT-17	Category 4	-	X	X	X	3
	CCAT-21	Category 3	-	X	X	-	2
	CHST-08	Category 4	-	X	X	X	3

* Cave could not be accessed for health and safety reasons, and therefore only subject to external monitoring techniques.

Appendix Table 3: Number of scats collected during each monitoring survey

Area	Cave ID	Trip 6 Jan/Feb 2023	Trip 7 Apr 2023	Trip 8 Jun/Jul 2023	Trip 9 Sep 2023	Total Scats Collected
Western Ridge	CWER-01	0	0	8	0	8
	CWER-03	71	0	1	0	72
	CWER-04	0	0	0	0	0
	CWER-05	-	0	0	0	0
	CWER-07	0	0	0	0	0
	CWER-09	0	0	0	0	0
	CWER-10	0	0	0	0	0
	CWER-14	0	0	0	0	0
	CWER-17	0	0	8	15	23
	CWER-20	-	0	0	0	0
Jimblebar/ Ninga	CJIM-01	0	0	0	0	0
	CJIM-03	0	0	0	0	0
	CJIM-04	0	0	0	0	0
	CJIM-05	0	0	0	0	0
	CJIM-06	0	0	0	0	0
	CJIM-07	0	0	0	0	0
	CJIM-08	0	0	0	0	0
	CJIM-09	-	0	0	0	0
	CJIM-14	0	0	0	0	0
	CJIM-15	0	0	0	0	0
	CJIM-16	0	0	0	0	0
	CJIM-17	0	0	0	0	0
	CJIM-18	0	0	0	0	0
	CJIM-20	-	-	0	0	0
	CNIN-01	0	5*	71 genetics 150 dietary	101	327
	CNIN-02	0	0	0	0	0
	CNIN-03	2	0	0	10	12
	CNIN-13	0	0	12	20	32
Cathedral Gorge	CCAT-01	-	0	0	0	0
	CCAT-02	-	0	0	0	0
	CCAT-06	0	0	0	0	0
	CCAT-09	-	0	0	0	0

Area	Cave ID	Trip 6 Jan/Feb 2023	Trip 7 Apr 2023	Trip 8 Jun/Jul 2023	Trip 9 Sep 2023	Total Scats Collected
	CCAT-10	-	0	0	-	0
	CCAT-13	-	0	0	0	0
	CCAT-14	-	0	0	0	0
	CCAT-17	-	0	0	0	0
	CCAT-21	-	0	0	-	0
	CHST-08	-	0	0	0	0
Total		73	5	250	146	474

Note: '-' denotes caves that were not visited during a given monitoring survey; '*' denotes scats collected but not from black scat sheets.

Appendix Table 4: Location and deployment information of the microclimate loggers

Area	Cave ID	Total Nights	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
			2022				2023								
Jimblebar Ninga	CJIM-01	371													
	CJIM-03	371													
	CJIM-04	298													
	CJIM-05	369													
	CJIM-06	363													
	CJIM-07	289													
	CJIM-08	369													
	CJIM-09	142													
	CJIM-14	362													
	CJIM-15	369													
	CJIM-16	371													
	CJIM-17	362													
	CJIM-18	344													
	CJIM-20	335													
	CNIN-01	299													
	CNIN-02	369													
	CNIN-03	370													
	CNIN-13	370													
Western Ridge	CWER-01	367													
	CWER-03	368													
	CWER-04	366													
	CWER-05	366													
	CWER-07	367													
	CWER-09	367													
	CWER-10	367													
	CWER-14	366													
	CWER-17	366													
	CWER-20	367													
Cathedral Gorge	CCAT-01	297													
	CCAT-02	369													
	CCAT-06	372													
	CCAT-09	358													
	CCAT-10	0													

Area	Cave ID	Total Nights	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
			2022				2023								
	CCAT-13	140													
	CCAT-14	300													
	CCAT-17	357													
	CCAT-21	247													
	CHST-08	365													

Note: The green illustrates the period microclimate loggers were deployed and recording data, the grey box illustrates the period during which data was not available due to technical difficulties.

^ Microclimate monitoring was not continued due to access constraints, i.e. the entry to the cave is high and the roosting area cannot be safely accessed

Appendix Table 5: Monitoring constraints and limitations

Potential limitation or constraint	Constraint	Applicability to this survey
Sources/ availability of data and information (recent or historic) and availability of contextual information	No	Sufficient information required for this report was available.
Competency/ experience of the survey team	No	The field personnel involved in the survey are experienced in undertaking fauna surveys within the Pilbara region, including targeted surveys for the ghost bat. The team lead, Chris Knuckey is highly experienced in undertaking ghost bat monitoring and targeted surveys. Bob Bullen, a recognised bat expert, assisted with analysis of ultrasonic recordings.
Scope (faunal groups sampled and whether any constraints affect this)	No	The scope was a ghost bat monitoring program and was conducted within that framework. Multiple techniques were employed to detect and monitor the species such as cave assessments, scat monitoring, ultrasonic recording, video recording, genetic analysis, dietary analysis, hormone analysis and GPS tracking (where possible).
Timing, weather, and season	No	Monitoring surveys occurred over appropriate or optimal periods for sampling the target species. No other weather or seasonality constraints or limitations were identified during the surveys.
Disturbances (e.g., fire or flood)	No	No disturbances such as fire or flood affected the surveys.
Proportion of fauna identified	No	All fauna observed during were identified to species level. Species identification of fauna recorded via camera traps and SongMeter ultrasonic recorders were able to be accurately identified with the assistance of technical personnel with relevant expertise.
Adequacy of the survey intensity and proportion of the survey achieved	No	The monitoring was undertaken between September 2022 and September 2023 as proposed.

Potential limitation or constraint	Constraint	Applicability to this survey
Problems with data and analysis, including sampling bias	Partial	A number of issues were encountered with the microclimate loggers and so a significant amount of data loss occurred. In July 2023, the logger in CNIN-03 was unable to be located, a replacement logger was installed but a period of data was lost. As CJIM-09 had not been visited since September 2022, when visited in April 2023 the battery was found to be dead and so data was only available from April 2023 onwards. In April 2023 the logger at CCAT-10 was found to have been moved outside the cave (presumably by animals) and was non-functional, assumed to be damaged from exposure to rain. A number of other caves also suffered from data loss due to various technical issues (mostly with battery health and the corruption of data). Some issues were experienced with the infra-red cameras (some were knocked over multiple times by animals, and spider-webs came to cover some lenses) (Table 2.4). Nonetheless, these issues were not considered to be a significant constraint as monitoring is ongoing.

Appendix E: Scat Monitoring Results

Appendix Table 6: Scat deposited during each monitoring period

Area	Cave ID	Trip 6 Jan/Feb 2023	Trip 7 Apr 2023	Trip 8 Jun/Jul 2023	Trip 9 Sep 2023
Western Ridge	CWER-01	0	0	8 (0.34)	0
	CWER-03	200 (0.92)	0	1 (0.12)	0
	CWER-04	0	0	0	0
	CWER-05	-	0	0	0
	CWER-07	0	0	0	0
	CWER-09	0	0	0	0
	CWER-10	0	0	0	0
	CWER-14	0	0	0	0
	CWER-16*	-	-	-	-
	CWER-17	0	0	8 (0.34)	15 (0.46)
	CWER-20	0	0	0	0
Jimblebar/Ninga	CJIM-01	0	0	0	0
	CJIM-03	0	0	0	0
	CJIM-04	0	0	0	0
	CJIM-05	0	0	0	0
	CJIM-06	0	0	0	0
	CJIM-07	0	0	0	0
	CJIM-08	0	0	0	0
	CJIM-09	-	0	0	0
	CJIM-14	0	0	0	0
	CJIM-15	0	0	0	0
	CJIM-16	0	0	0	0
	CJIM-17	0	0	0	0
	CJIM-18	0	0	0	0
	CJIM-20	-	-	0	0
	CNIN-01	0	5 (0.1)	230 (1.83)	150 (1.45)
	CNIN-02	0	0	0	0
	CNIN-03	2 (0.12)	0	0	10 (0.38)
	CNIN-09*	-	-	-	-
	CNIN-13	0	0	12 (0.41)	50 (0.84)
Cathedral Gorge	CCAT-01	-	0	0	0
	CCAT-02	-	0	0	0
	CCAT-06	0	0	0	0
	CCAT-09	-	0	0	0

Area	Cave ID	Trip 6 Jan/Feb 2023	Trip 7 Apr 2023	Trip 8 Jun/Jul 2023	Trip 9 Sep 2023
	CCAT-10	-	0	0	-
	CCAT-13	-	0	0	0
	CCAT-14	-	0	0	0
	CCAT-17	-	0	0	0
	CCAT-21	-	0	0	-
	CHST-08	-	0	0	0

* Cave could not be accessed for health and safety reasons, and therefore only subject to external monitoring techniques. ^ Scats were recorded off sheet.

Appendix F: Hormone Analysis Results

Note: Gold coloured cells denote Intermediate progesterone (700-999 ng/g) levels and green coloured cells denote elevated progesterone (>1000 ng/g) levels. Blue coloured cells denote no elevated progesterone found in sample set.

Appendix Table 7: Hormone analysis

Cave ID	Date	Progesterone ng/g
CNIN-03	2/02/2023	1,176.284585
CNIN-03	2/02/2023	651.4285714
CWER-03	1/02/2023	198.3333333
CWER-03	1/02/2023	266.122449
CWER-03	1/02/2023	194.1422594
CWER-03	1/02/2023	215.5102041
CWER-03	1/02/2023	806.6115702
CWER-03	1/02/2023	248.1632653
CWER-03	1/02/2023	252.2821577
CWER-03	1/02/2023	200.7782101
CWER-03	1/02/2023	169.7478992
CWER-03	1/02/2023	110.6542056
CWER-03	1/02/2023	199.6039604
CWER-03	1/02/2023	287.6033058
CWER-03	1/02/2023	334.2574257
CWER-03	1/02/2023	1,184.962406
CWER-03	1/02/2023	800
CWER-03	1/02/2023	1,073.092369
CWER-03	1/02/2023	317.7419355
CWER-03	1/02/2023	180.8
CWER-03	1/02/2023	136
CWER-03	1/02/2023	733.3333333
CWER-03	1/02/2023	1,139.09465
CWER-03	1/02/2023	265.5737705
CWER-03	1/02/2023	243.5483871
CWER-03	1/02/2023	191.8032787
CWER-03	1/02/2023	1,064.516129
CWER-03	1/02/2023	996.2886598
CWER-03	1/02/2023	199.2337165
CWER-03	1/02/2023	928
CWER-03	1/02/2023	273.2743363
CWER-03	1/02/2023	200

Cave ID	Date	Progesterone ng/g
CWER-03	1/02/2023	270
CWER-03	1/02/2023	298.7755102
CWER-03	1/02/2023	253.9130435
CWER-03	1/02/2023	973.9130435
CWER-03	1/02/2023	121.6730038
CWER-03	1/02/2023	197.5903614
CWER-03	1/02/2023	230.2803738
CWER-03	1/02/2023	658.8235294
CWER-03	1/02/2023	316.9491525
CWER-03	1/02/2023	257.6271186
CWER-03	1/02/2023	166.4122137
CWER-03	1/02/2023	978.5123967
CWER-03	1/02/2023	203.0534351
CWER-03	1/02/2023	999.1561181
CWER-03	1/02/2023	304.8421053
CWER-03	1/02/2023	192.1259843
CWER-03	1/02/2023	105.6910569
CWER-03	1/02/2023	232.8888889
CWER-03	1/02/2023	315.8730159
CWER-03	1/02/2023	701.1235955
CWER-03	1/02/2023	585.5319149
CWER-03	1/02/2023	304.8387097
CWER-03	1/02/2023	1,167.835052
CWER-03	1/02/2023	227.9069767
CWER-03	1/02/2023	298.3606557
CWER-03	1/02/2023	731.4285714
CWER-03	1/02/2023	401.6393443
CWER-03	1/02/2023	233.5135135
CWER-03	1/02/2023	617.3228346
CWER-03	1/02/2023	1,064.516129
CWER-03	1/02/2023	195.1648352
CWER-03	1/02/2023	204.9180328
CWER-03	1/02/2023	321.6326531
CWER-03	1/02/2023	342.2053232
CWER-03	1/02/2023	521.2121212
CWER-03	1/02/2023	217.1875
CWER-03	1/02/2023	309.787234

Cave ID	Date	Progesterone ng/g
CWER-03	1/02/2023	146.0076046
CWER-03	1/02/2023	314.893617
CWER-03	1/02/2023	268.3127572
CWER-03	1/02/2023	272.9411765
CNIN-01	24/04/2023	130.7984791
CNIN-01	24/04/2023	99.2248062
CNIN-01	24/04/2023	80.3030303
CNIN-01	24/04/2023	145.4545455
CNIN-01	24/04/2023	220.9677419
CWER-03	29/06/2023	74.41860465
CNIN-01	2/07/2023	2,757.575758
CNIN-01	2/07/2023	137.1428571
CNIN-01	2/07/2023	120.610687
CNIN-01	2/07/2023	106.9767442
CNIN-01	2/07/2023	158.7548638
CNIN-01	2/07/2023	203.3333333
CNIN-01	2/07/2023	111.8773946
CNIN-01	2/07/2023	101.6129032
CNIN-01	2/07/2023	210.9375
CNIN-01	2/07/2023	115.2263374
CNIN-01	2/07/2023	148.6868687
CNIN-01	2/07/2023	92.06349206
CNIN-01	2/07/2023	213.559322
CNIN-01	2/07/2023	93.84615385
CNIN-01	2/07/2023	122.6168224
CNIN-01	2/07/2023	224.7011952
CNIN-01	2/07/2023	not enough
CNIN-01	2/07/2023	99.60474308
CNIN-01	2/07/2023	582.3754789
CNIN-01	2/07/2023	95.23809524
CNIN-01	2/07/2023	183.1932773
CNIN-01	2/07/2023	89.0625
CNIN-01	2/07/2023	117.3745174
CNIN-01	2/07/2023	210.7692308
CNIN-01	2/07/2023	108.8122605
CNIN-01	2/07/2023	103.875969
CNIN-01	2/07/2023	148.9878543

Cave ID	Date	Progesterone ng/g
CNIN-01	2/07/2023	156.4516129
CNIN-01	2/07/2023	137.9844961
CNIN-01	2/07/2023	122.1757322
CNIN-01	2/07/2023	3,697.478992
CNIN-01	2/07/2023	200.7843137
CNIN-01	2/07/2023	80.64516129
CNIN-01	2/07/2023	204.1493776
CNIN-01	2/07/2023	76.66666667
CNIN-01	2/07/2023	91.37931034
CNIN-01	2/07/2023	215
CNIN-01	2/07/2023	355.5555556
CNIN-01	2/07/2023	176.1904762
CNIN-01	2/07/2023	86.05577689
CNIN-01	2/07/2023	60.31746032
CNIN-01	2/07/2023	53.33333333
CNIN-01	2/07/2023	66.12903226
CNIN-01	2/07/2023	77.16535433
CNIN-01	2/07/2023	76.9874477
CNIN-01	2/07/2023	115.2
CNIN-01	2/07/2023	145.6
CNIN-01	2/07/2023	99.2
CNIN-01	2/07/2023	140.9090909
CNIN-01	2/07/2023	215.5102041
CNIN-01	2/07/2023	138.3333333
CNIN-01	2/07/2023	291.6030534
CNIN-01	2/07/2023	152.1568627
CNIN-01	2/07/2023	125.8964143
CNIN-01	2/07/2023	106.122449
CNIN-01	2/07/2023	122.8215768
CNIN-01	2/07/2023	145.1476793
CNIN-01	2/07/2023	219.6078431
CNIN-01	2/07/2023	149.8069498
CNIN-01	2/07/2023	134.3629344
CNIN-01	2/07/2023	2,821.576763
CNIN-01	2/07/2023	85.48387097
CNIN-01	2/07/2023	81.01265823
CNIN-01	2/07/2023	155.8232932

Cave ID	Date	Progesterone ng/g
CNIN-01	2/07/2023	137.6
CNIN-01	2/07/2023	83.65019011
CNIN-01	2/07/2023	204.0816327
CNIN-01	2/07/2023	154.3071161
CNIN-01	2/07/2023	118.1818182
CNIN-01	2/07/2023	90.07633588
CNIN-01	2/07/2023	130.0411523
CWER-01	30/06/2023	1,086.419753
CWER-01	30/06/2023	3,566.26506
CWER-01	30/06/2023	128.3950617
CWER-01	30/06/2023	900.7633588
CWER-01	30/06/2023	2,415.686275
CWER-01	30/06/2023	778.2101167
CWER-01	30/06/2023	1,226.05364
CWER-01	30/06/2023	890.5660377
CWER-17	30/06/2023	not enough
CWER-17	30/06/2023	117.7358491
CWER-17	30/06/2023	175.8024691
CWER-17	30/06/2023	204.5454545
CWER-17	30/06/2023	171.2
CWER-17	30/06/2023	203.125
CWER-17	30/06/2023	207.4074074
CWER-17	30/06/2023	161.6326531
CNIN-13	2/07/2023	255.513308
CNIN-13	2/07/2023	336.9294606
CNIN-13	2/07/2023	258.8235294
CNIN-13	2/07/2023	186.440678
CNIN-13	2/07/2023	288.4120172
CNIN-13	2/07/2023	242.9752066
CNIN-13	2/07/2023	274.5098039
CNIN-13	2/07/2023	121.5384615
CNIN-13	2/07/2023	318.3505155
CNIN-13	2/07/2023	not enough
CNIN-13	2/07/2023	190.7949791
CNIN-13	2/07/2023	218.8679245
CNIN-01	11/09/2023	135.5648536
CNIN-01	11/09/2023	82.30452675

Cave ID	Date	Progesterone ng/g
CNIN-01	11/09/2023	127.4900398
CNIN-01	11/09/2023	196.9924812
CNIN-01	11/09/2023	64.36781609
CNIN-01	11/09/2023	319.0661479
CNIN-01	11/09/2023	9,225.806452
CNIN-01	11/09/2023	2,564.885496
CNIN-01	11/09/2023	151.4893617
CNIN-01	11/09/2023	180
CNIN-01	11/09/2023	132.8125
CNIN-01	11/09/2023	207.4074074
CNIN-01	11/09/2023	278.4
CNIN-01	11/09/2023	286.695279
CNIN-01	11/09/2023	156.7346939
CNIN-01	11/09/2023	130.1587302
CNIN-01	11/09/2023	176.2711864
CNIN-01	11/09/2023	181.9742489
CNIN-01	11/09/2023	143.0894309
CNIN-01	11/09/2023	not enough
CNIN-01	11/09/2023	235.2059925
CNIN-01	11/09/2023	4045.283019
CNIN-01	11/09/2023	1614.953271
CNIN-01	11/09/2023	75.75757576
CNIN-01	11/09/2023	156.6539924
CNIN-01	11/09/2023	101.6129032
CNIN-01	11/09/2023	191.0204082
CNIN-01	11/09/2023	84.6473029
CNIN-01	11/09/2023	150.8196721
CNIN-01	11/09/2023	143.6293436
CNIN-01	11/09/2023	101.9305019
CNIN-01	11/09/2023	191.4529915
CNIN-01	11/09/2023	257.0281124
CNIN-01	11/09/2023	502.8571429
CNIN-01	11/09/2023	134.8484848
CNIN-01	11/09/2023	106.6666667
CNIN-01	11/09/2023	169.0566038
CNIN-01	11/09/2023	166.1538462
CNIN-01	11/09/2023	125.7142857

Cave ID	Date	Progesterone ng/g
CNIN-01	11/09/2023	54.6875
CNIN-01	11/09/2023	372.244898
CNIN-01	11/09/2023	166.1538462
CNIN-01	11/09/2023	126.5822785
CNIN-01	11/09/2023	142.6229508
CNIN-01	11/09/2023	143.9393939
CNIN-01	11/09/2023	140.9961686
CNIN-01	11/09/2023	259.9156118
CNIN-01	11/09/2023	168.2539683
CNIN-01	11/09/2023	271.1864407
CNIN-01	11/09/2023	101.5873016
CNIN-01	11/09/2023	211.9521912
CNIN-01	11/09/2023	217.4273859
CNIN-01	11/09/2023	144.5378151
CNIN-01	11/09/2023	204.5112782
CNIN-01	11/09/2023	159.3625498
CNIN-01	11/09/2023	86.8852459
CNIN-01	11/09/2023	371.7647059
CNIN-01	11/09/2023	63.878327
CNIN-01	11/09/2023	247.1910112
CNIN-01	11/09/2023	225
CNIN-01	11/09/2023	258.0645161
CNIN-01	11/09/2023	255.7620818
CNIN-01	11/09/2023	155.8232932
CNIN-01	11/09/2023	117.6470588
CNIN-01	11/09/2023	122.3140496
CNIN-01	11/09/2023	66.40316206
CNIN-01	11/09/2023	248.5596708
CNIN-01	11/09/2023	314.2857143
CNIN-01	11/09/2023	230.5343511
CNIN-01	11/09/2023	223.9382239
CNIN-01	11/09/2023	93.38521401
CNIN-01	11/09/2023	62.29508197
CNIN-01	11/09/2023	4,034.782609
CNIN-01	11/09/2023	36.94779116
CNIN-01	11/09/2023	137.2881356
CNIN-01	11/09/2023	5,386.138614

Cave ID	Date	Progesterone ng/g
CNIN-01	11/09/2023	139.9176955
CNIN-01	11/09/2023	158.7548638
CNIN-01	11/09/2023	56.91699605
CNIN-01	11/09/2023	104.6875
CNIN-01	11/09/2023	109.5238095
CNIN-01	11/09/2023	208.7649402
CNIN-01	11/09/2023	336.121673
CNIN-01	11/09/2023	221.2121212
CNIN-01	11/09/2023	356.7099567
CNIN-01	11/09/2023	168.1632653
CNIN-01	11/09/2023	239.688716
CNIN-01	11/09/2023	181.0699588
CNIN-01	11/09/2023	411.7647059
CNIN-01	11/09/2023	249.1803279
CNIN-01	11/09/2023	227.8884462
CNIN-01	11/09/2023	601.5037594
CNIN-01	11/09/2023	146.7741935
CNIN-01	11/09/2023	115.1750973
CNIN-01	11/09/2023	157.0247934
CNIN-01	11/09/2023	229.4573643
CNIN-01	11/09/2023	203.2258065
CNIN-01	11/09/2023	242.9752066
CNIN-01	11/09/2023	477.6119403
CNIN-01	11/09/2023	4,702.290076
CNIN-01	11/09/2023	253.7815126
CNIN-03	11/09/2023	344.8275862
CNIN-03	11/09/2023	307.8125
CNIN-03	11/09/2023	4,273.504274
CNIN-03	11/09/2023	172.1311475
CNIN-03	11/09/2023	154.6218487
CNIN-03	11/09/2023	338.8429752
CNIN-03	11/09/2023	443.75
CNIN-03	11/09/2023	162.248996
CNIN-03	11/09/2023	842.1052632
CNIN-03	11/09/2023	3,256.637168
CNIN-13	11/09/2023	88.66995074
CNIN-13	11/09/2023	82.01680672

Cave ID	Date	Progesterone ng/g
CNIN-13	11/09/2023	99.59183673
CNIN-13	11/09/2023	127.510917
CNIN-13	11/09/2023	169.9115044
CNIN-13	11/09/2023	141.509434
CNIN-13	11/09/2023	201.8018018
CNIN-13	11/09/2023	135.8139535
CNIN-13	11/09/2023	290
CNIN-13	11/09/2023	264.9122807
CNIN-13	11/09/2023	158.974359
CNIN-13	11/09/2023	345.2991453
CNIN-13	11/09/2023	266.1016949
CNIN-13	11/09/2023	172.9292929
CNIN-13	11/09/2023	200.8888889
CNIN-13	11/09/2023	170.2811245
CNIN-13	11/09/2023	235.1851852
CNIN-13	11/09/2023	142.1487603
CNIN-13	11/09/2023	175.1004016
CNIN-13	11/09/2023	171.1790393
CWER-17	8/09/2023	423.628692
CWER-17	8/09/2023	333.9130435
CWER-17	8/09/2023	266.1016949
CWER-17	8/09/2023	156.1904762
CWER-17	8/09/2023	155.0239234
CWER-17	8/09/2023	51.06382979
CWER-17	8/09/2023	365.4545455
CWER-17	8/09/2023	173.0769231
CWER-17	8/09/2023	80
CWER-17	8/09/2023	120.9677419
CWER-17	8/09/2023	199.2094862
CWER-17	8/09/2023	178.4037559
CWER-17	8/09/2023	454.0540541
CWER-17	8/09/2023	334.2222222
CWER-17	8/09/2023	143.3333333

Appendix G: DBCA Genetic Analysis Report



Department of **Biodiversity,
Conservation and Attractions**

Ghost bat (*Macroderma gigas*) genetic monitoring: Western
Ridge-Jimblebar 2022

Version: 1.1

Approved by:

Last Updated: 24 May 2023

Custodian: Kym Ottewell

Review date:

Version number	Date approved DD/MM/YYYY	Approved by	Brief Description
1.1	24/05/2023		Draft report presented to Biologic for comment
1.2	29/05/2023		Accepted changes and changed figures to reflect updated roost names



Biodiversity and Conservation Science

Ghost bat (*Macroderma gigas*) genetic monitoring: Western Ridge-Jimblebar February -September 2022

Diana Prada, Melissa Millar, and Kym Ottewell

Draft report to Biologic Environmental Survey
May 2023



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May 2023

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This document is available in alternative formats on request.

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Acknowledgments

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Summary

This project describes the annualised patterns of spatial and temporal cave use by ghost bats in the Western Ridge-Jimblebar precinct (eastern Hamersley Range, Pilbara, Western Australia).

In this report:

- 155 *Macroderma gigas* faecal DNA samples were analysed from nine roosts located within the Western Ridge-Jimblebar region, Pilbara, Western Australia. Sampling occurred between February and September 2022.
- Samples were genotyped at either 46 or 49 SNP loci using high-throughput SNP genotyping.
- Due to the quality of the data, conservative filters were applied to reach a minimum number of individuals in the surveyed area.
- Useable genotypes were obtained from 108 samples and 11 unique individuals were identified (four males, seven females).
- Ten bats were detected for the first time, and one bat (135 Female) was a 'recaptured' bat, first detected in 2016 (survey database 2015 – 2022).
- Three individuals were captured on multiple occasions within the survey interval (135 Female, 856 Female, and 874 Male) indicating these are putative resident bats.
- Recaptured bats showed fidelity to clusters of roosts that were <10 km apart. Most recaptured bats in this study used one or two roosts.
- Pairwise relatedness values between the identified bats were low. No first-degree relationships (parent-offspring or full-siblings) were observed from the current dataset.

1 Introduction

The ghost bat (*Macroderma gigas*) is a monotypic bat species native to the Pilbara and Kimberley regions of Western Australia (WA), the Northern Territory (NT) and eastern Australia. Throughout northern Australia (QLD, NT, northern WA), they are coastal and occur up to 400 km inland, generally north of the Tropic of Capricorn. They appear to occupy a wide range of habitats from rainforest, monsoon and vine scrub in the tropics, to open woodlands and arid areas, such as the Pilbara, which is geographically isolated from extant northern Australian populations (and the historical central Australian populations) by extensive sandy deserts. The ghost bat is an obligate troglodyte, and survival is critically dependent on finding natural roosts in caves, crevices, deep overhangs and artificial roosts such as abandoned mines (Hall et al., 1997). Populations of this species appear to have regionally centralised maternity roosts that are genetically isolated from each other (Worthington Wilmer et al., 1994). The species is characterised by high maternal philopatry and male-biased dispersal (Worthington Wilmer et al., 1994). Populations are known to disperse in the non-breeding (dry) season (G, J Toop, 1985; G.J Toop, 1979).

Genetic monitoring of ghost bat roosts has been ongoing since 2015 in different areas of the Pilbara (Ottewell et al., 2019, 2021; Spencer & Tedeschi, 2016). In this report we undertake analyses for Western Ridge-Jimblebar ghost bat faecal samples collected in 2022 using our recently established high-throughput SNP genotyping method. Importantly, the samples for this project are separated in two cohorts; samples collected in February 2022 were genotyped at 46 SNP sites while samples collected in June and September were genotyped at 49 SNP sites.

1.1 Study aims

This project reports on genetic monitoring of the ghost bat population within the Western Ridge-Jimblebar mining precinct for the February – September 2022 survey period.

The study aims were to:

1. Genotype faecal samples and identify unique genetic profiles.
2. Identify unique individuals from genotype data and undertake molecular sexing of unique individuals.
3. Identify 'recaptures' by comparing newly identified individuals to the Pilbara ghost bat genetic profile database, collated by DBCA.
4. Spatial-temporal analyses:
 - a. Identification of putative resident vs vagrant ghost bats in the study area.
 - b. Description of temporal and spatial roost occupancy patterns.
5. Population genetic analyses:
 - a. Estimate genetic diversity and inbreeding levels.
 - b. Assess genetic relatedness and relationships amongst identified individuals.

2 Materials and Methods

2.1 Sampling locations and material

A total of 155 ghost bat scat samples were collected from nine roosts within the Western Ridge mining precinct between February-September 2022 by Biologic Environmental Survey staff (Figure 1).

Up to 20 fresh ghost bat scats were collected from tarpaulins that had been placed on roost floors for several months. Each scat was collected into an individual envelope and kept frozen until DNA extraction.

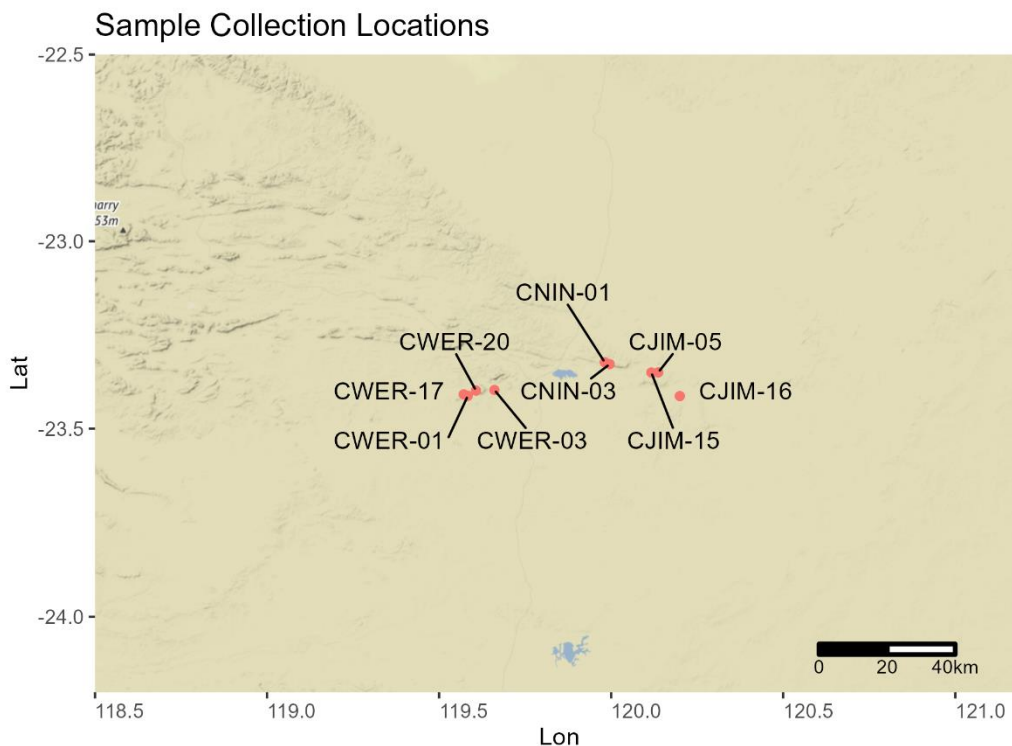


Figure 1. Map of roosts surveyed in the Western Ridge region between February-September 2022

2.2 DNA extraction and SNP genotyping

DNA extraction was carried out in the Biologic laboratory following methods in Ottewell et al. (2022). Extracted DNA samples were delivered to the Department of Biodiversity, Conservation and Attractions (DBCA) laboratory for molecular sexing and preparation for SNP genotyping at the Australian Genome Research Facility, Brisbane (AGRF).

All 155 samples were genotyped using a custom-designed multiplexed panel of single nucleotide polymorphism (SNP) markers specifically designed to target highly polymorphic SNP loci in Pilbara populations to enable robust identification of

individuals from non-invasive samples (Thavornkanlapachai et al., n.d.). Due to ongoing refinement of the SNP array and sample submission timing, samples collected in February 2022 ($n = 25$) were genotyped at 46 SNP markers while the remaining samples were genotyped at 49 SNPs. Sixty μl of each faecal DNA sample was sub-sampled from 100 μl of DNA extract and concentrated down to 20 μl for genotyping. PCR amplification and extension reactions carried out by AGRF were performed using the iPLEX Gold Reagent Kit with 1 μl of the concentrated DNA extract. Resultant SNP genotypes were identified by mass spectrometry and called using MassARRAY TYPERVIEWER 4.0 software (Agena Bioscience). Nineteen samples were re-genotyped to allow calculation of allelic dropout and false allele error rates.

2.3 Estimation of the number of unique individuals

MassARRAY SNP results were processed in a custom R package ‘ScatMatch’ (Huntley, 2021). The package groups scat genetic profiles based on their pairwise similarity across loci. It allows the filtering of SNP genotyping data based on user defined amplification thresholds across samples and loci, and the subsequent evaluation of putative number of unique individuals under different mismatch thresholds (h). Genotyping errors are frequently observed in studies using scat DNA due to the low quality and quantity of DNA sourced from these samples (Knapp et al., 2009; Taberlet et al., 1999) leading to genetic mismatches between samples. Choosing analysis parameters that are either too stringent (e.g., high levels of amplification and little tolerance for mismatches) or too relaxed would lead to the underestimation or overestimation of unique number of individuals in the dataset, respectively.

We explored the putative number of individuals under the following data subsets:

- a) Amplification across samples $\geq 70\%$ and amplification across loci $\geq 80\%$
- b) Amplification across samples $\geq 77\%$ and amplification across loci $\geq 80\%$
- c) Amplification across samples $\geq 80\%$ and amplification across loci $\geq 80\%$

The level of filtering and the mismatch threshold was chosen by evaluating the number of groups under different values of h (left panel, Figure 2) and the distribution of SNP differences within and between individuals, represented as a binomial distribution (right panel, Figure 2). The clearer separation between these two peaks in the binomial distribution, the greater certainty on the “true” number of unique individuals represented in the dataset. A detailed explanation of these steps has been provided previously in Ottewell et al. (2022).

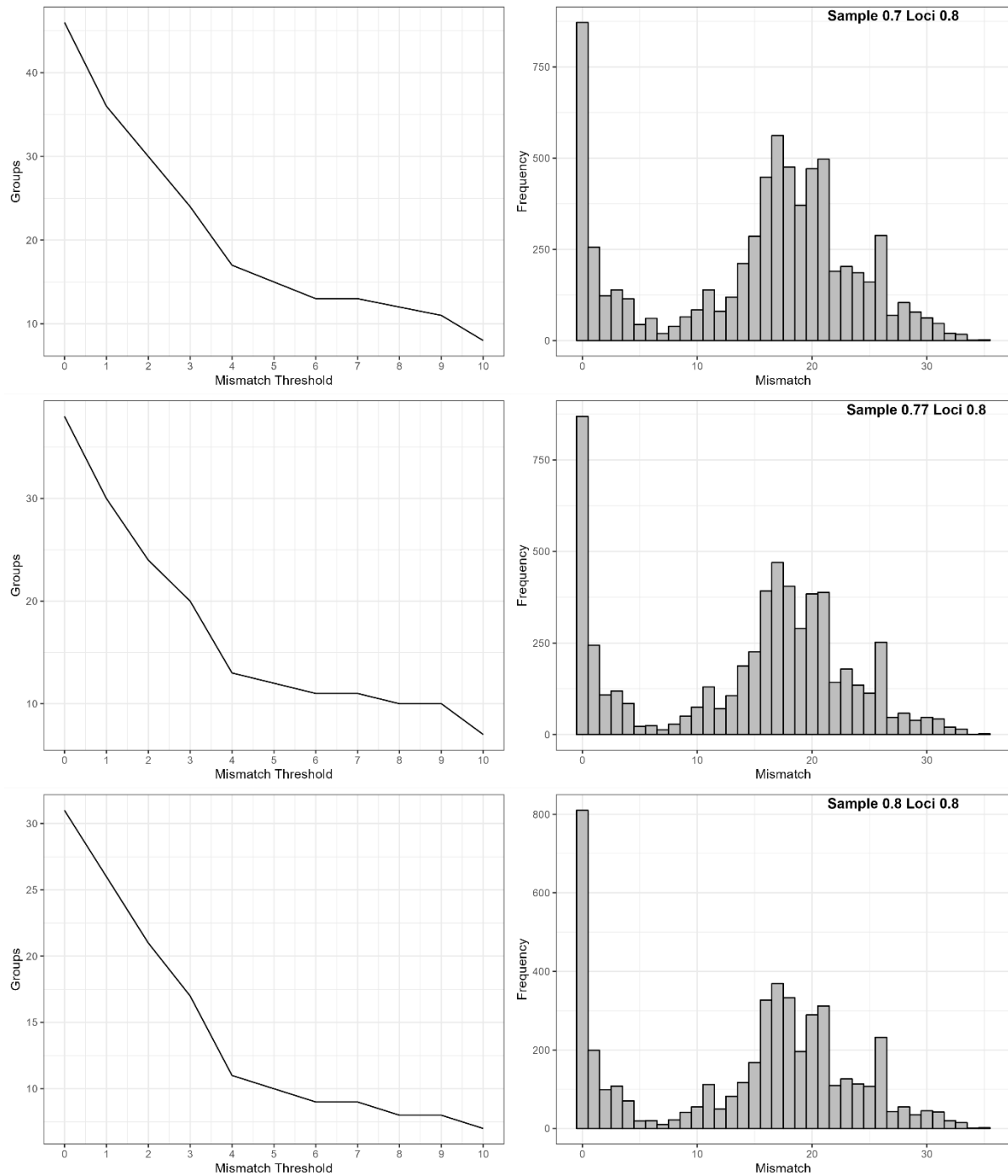


Figure 2. Assessment of the distribution of allelic mismatches with different sample and locus amplification rate filters (70%, 77%, and 80%) and evaluation of the mismatch threshold (h) used to group *Macroderma gigas* scats from the same individuals. Left: Elbow graph showing the number of individuals (Groups) identified with increasing number of allelic mismatches (Mismatch Threshold). Right: Binomial frequency distribution of pairwise allelic mismatches within and between samples.

For this dataset we decided to keep samples with an amplification success rate of $\geq 77\%$ and loci with an amplification rate $\geq 80\%$ and set up a mismatch threshold (h) of six. The chosen mismatch threshold provided a clear separation between the distribution of mismatches between and within samples (Figure 2 middle pane). This threshold is higher than normally used (typical $h = 2-4$) and may have the effect of clustering scats from very closely related individuals and therefore underestimating the total number of bats present. We took this conservative approach to reduce the effect of over-splitting scat genotypes based on genotyping errors rather than true genetic differences which may have resulted in an over-estimation of the number of bats present.

2.4 Molecular sexing

Molecular sexing of scat samples was carried out using four custom-designed ghost bat sex-linked customised primers and probes (DDX3Y, SRY, Zfy, and Zfx) arranged in a real-time PCR multiplex (Ottewell et al., 2020). Primers were amplified in 10 μ l reactions using the PrimeTime™ Gene Expression Master Mix (Cat No: 1055772) following the manufacturer's instructions with an annealing temperature of 60°C, 40 amplification cycles and 4 μ l of unconcentrated DNA. The reactions were run on the CFX96™ Real-Time System C1000 Touch Thermal Cycle (BIO-RAD, Singapore) and analysed in BioRad CFX Maestro software (BIO-RAD, Singapore). We repeated qPCR twice to ensure consistency in sex identification. Sex is confidently allocated if all three Y-linked markers produced relative fluorescent units (RFUs) ≥ 50 , all markers produced the same result and are consistent across multiple scats from the same individual, and Y-linked marker RFU to the total RFU ratios are above 0.1 (DDX3Y, SRY) and 0.3 (Zfy) for males. Where inconsistencies are present (failed marker, mismatch between different scats) sex is indicated as “most likely” if 2 of 3 sex-linked markers amplify or “undetermined” if ≤ 1 marker amplify or if results are mismatched between scats from the same individual.

2.5 Assessment of sampling effort

The rate of accumulation of new individuals with increasing sample size was assessed using rarefaction analysis. A single, sample-based rarefaction curve was calculated in the software EstimateS v9.1.0 (Colwell et al., 2012). We used non-parametric extrapolation and the classic formula for Chao 1 and Chao 2 instead of the default bias-corrected formula because the coefficient of variation (CV) of the abundance was > 0.5 . To explore the trajectory of the rarefaction curve, sampling effort was set to roughly double the filtered sample size ($n = 216$ scats analysed in total).

2.6 Identification of 'recaptures' and spatial-temporal movements.

The identified individuals at Western Ridge-Jimblebar were compared against the DBCA scat genotype database using our custom R package, ScatMatch (Huntley, 2021). The parameters for the comparison were set up at a sample amplification rate $\geq 70\%$, loci amplification rate $\geq 80\%$ and a mismatch threshold of six to allow matching to lower quality samples present in the database. The genotypes of matched samples were closely inspected to confirm confidence in the identification of recaptures.

To explore movements between recaptures, we calculated the distance between detections of the same individuals at different dates. Hence, if an individual was detected in the same roost across two months this corresponded to a distance of 0 km.

2.7 Population genetic analyses

Population genetic diversity was calculated based on the total number of individuals detected in this study. Calculations were based on the 37 loci shared by all individuals after filtering. Summary population genetic diversity statistics, including observed (H_o) and expected heterozygosity (H_e), number of alleles (N_a) and the inbreeding coefficient (F_{is}) were calculated in GENALEX v6.5 (Peakall & Smouse, 2012). GENALEX was also used to estimate the mean relatedness (r) and pairwise genetic relatedness using the Ritland method (Ritland, 1996). Due to the high proportion of new individuals being located in the same roost (9/11 individuals) we were unable to undertake spatial autocorrelation analysis, as has been provided in other reports.

The program NeEstimator (Do et al., 2014) was used to estimate the contemporary effective population size (N_e) using the Linkage Disequilibrium method (Waples, 2006). We retained alleles with ≥ 0.05 allele frequency for this analysis. The program also implements a parametric method to calculate the 95% confidence intervals of N_e estimates (Jones et al., 2016).

3 Results

3.1 Genotyping success and number of unique individuals

The average amplification success rate of the overall batch of samples, including replicates, was $75 \pm 0.02\%$. No samples were excluded due to total amplification failure. The average rate of allelic drop out was 0.15 ± 0.01 , and the false allele error was 0.06 ± 0.006 . Sample details are summarised in appendix 1.

The filtered dataset included 108 samples in total. For samples collected in February 2022, 37 loci passed the filtering thresholds while for samples collected later that year, the filtered data consisted of 42 loci. A total of 11 unique individuals, seven females and four males, were identified from five out of the nine surveyed roosts.

The rate of accumulation of new individuals (Figure 3) indicated that the observed number of individuals was congruent with the expected value given the sampling effort. The analysis also suggests that further sampling would be required to detect additional members of the local population.

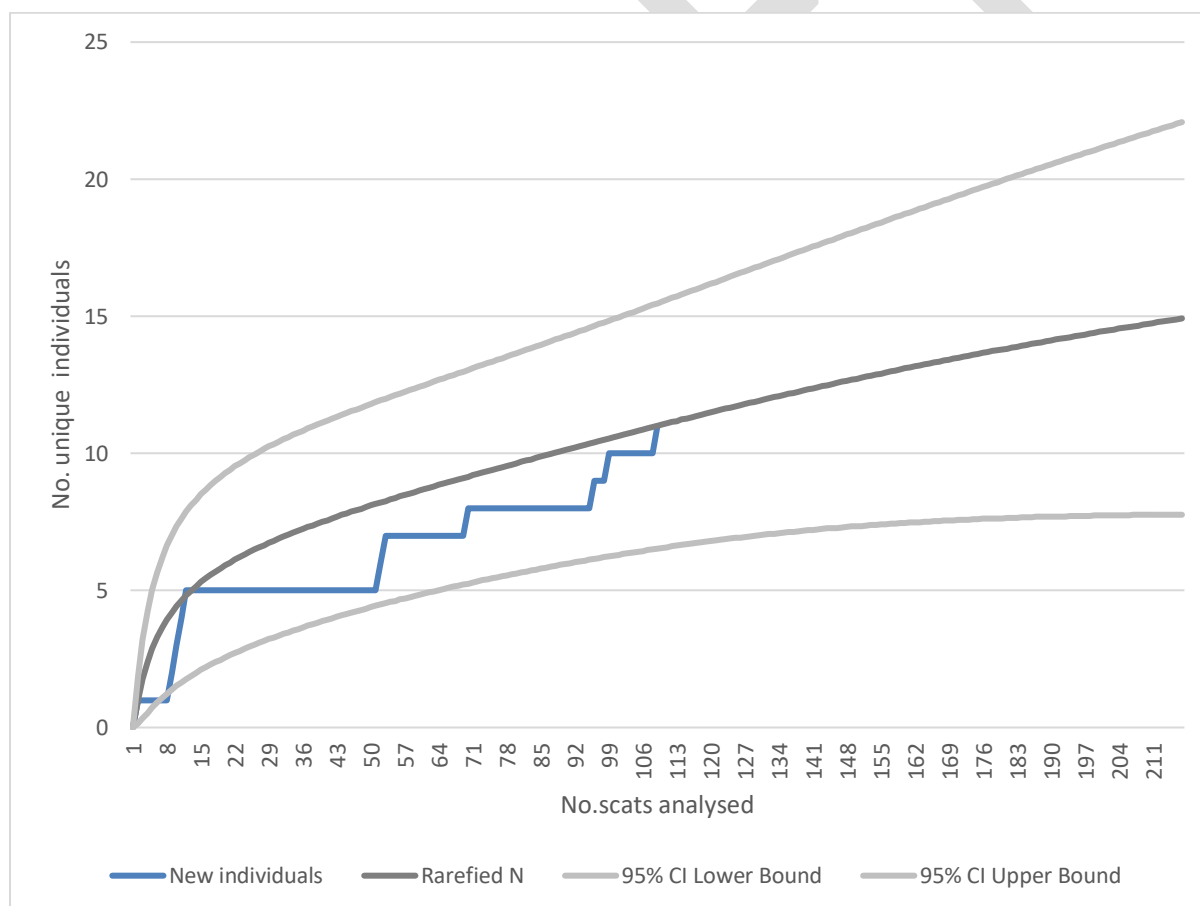


Figure 3. Sample rarefaction curve showing the accumulation of new genotypes with increasing numbers of scat samples analysed at Western Ridge-Jimblebar.

3.2 Number of unique individuals per roost

The number of unique individuals detected per roost varied between zero and nine, with the greatest number of individuals detected in cave CWER-03 (Figure 4). Samples collected from roosts CJIM-15, CNIN-01, CWER-01, CWER-20, and CJIM-05 did not produce any genotypes above the filtering thresholds.

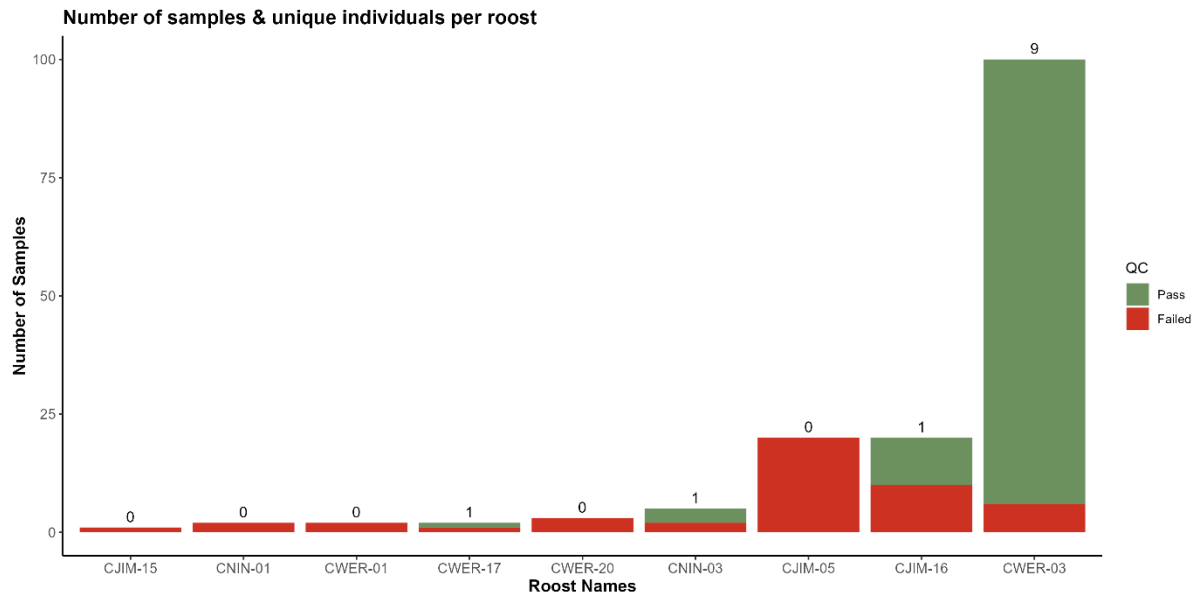


Figure 4. Number of samples collected per roost with the number of unique ghost bat genotypes identified shown as a number above each bar. Colours correspond to the number of samples that passed or failed the amplification threshold (QC).

Once data was stratified by sex (Figure 5), females were identified from roost CWER-03 and the remaining three roosts were represented by male individuals.

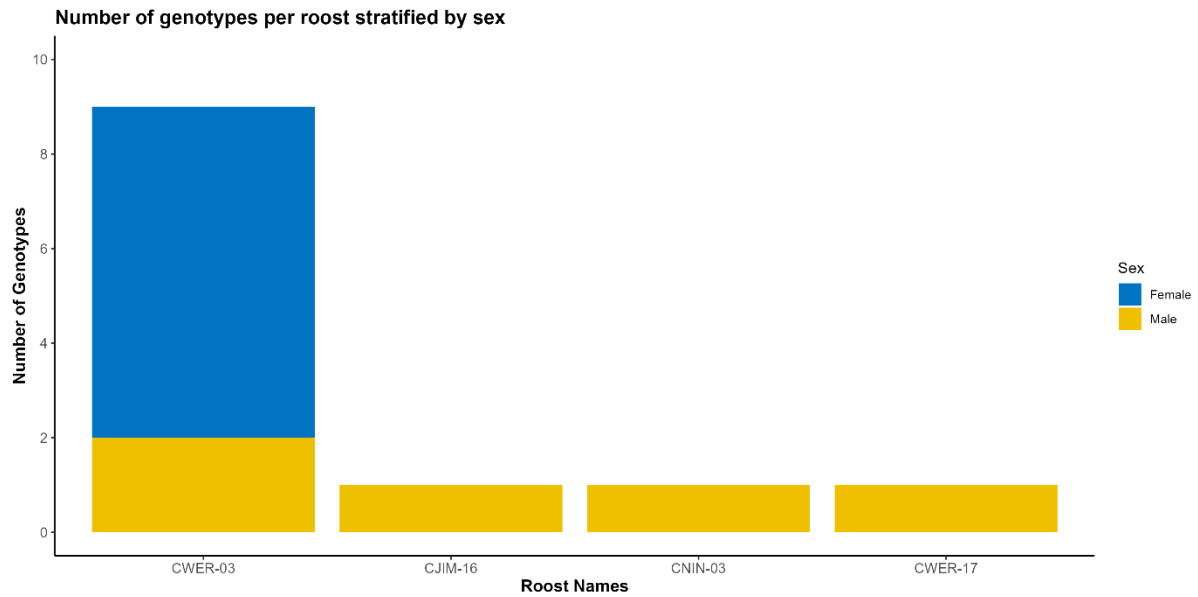


Figure 5. Number of ghost bat individuals per roost in 2022, stratified by sex.

3.3 Numbers of unique individuals per month

Samples within the dataset represent collection across three discontinuous months (Table 1). Samples collected in February resulted in the identification of most unique individuals followed by samples collected in June 2022.

Table 1. Number of ghost bat individuals detected in each cave at different sampling times in 2022 at Western Ridge-Jimblebar.

Roost	Feb-2022	Jun-2022	Sep-2022	Unique ind.
CJIM-16			1	1
CNIN-03		1		1
CWER-03	7	4		9
CWER-17			1	1

3.4 Recaptures and spatial-temporal patterns of roost use

Of the 11 unique ghost bats detected in this dataset, one female (Genotype 135) was identified as a 'recapture' from previous years (2016, 2017, 2020). An additional two new individuals (F856, M874) were identified as recaptures across the nine months of this study. Individual 135 has been detected at two roosts with a maximum distance between sites of 8 km. Individual 874 was detected at two roosts 9 km apart, while individual 856 was detected in the same roost for a total distance travelled of 0 km. Detection histories for each individual are summarised in table 2.

Table 2. Detection history (date and location) of unique ghost bat identified as 'recaptures' in the current study.

Genotype	Sex	Roost	2016	2017	2020	2022			
			5-May	20-Oct	25-Aug	10-Oct	26-Feb	11-Jun	7-Sep
135	Female	CWER-03	●		●	●	●	●	
		CWER-01	●	●					
856	Female	CWER-03					●	●	
874	Male	CWER-03						●	
		CWER-17							●

3.5 Genetic diversity and effective population size

We compared the genetic diversity indices derived from this study with those obtained from South Flank, which is the closest region to Western Ridge-Jimblebar (Table 3). Since samples in this report were genotyped at different number of SNPs, analyses were based on the 37 overlapping loci.

In general, genetic diversity indices from this study were lower to other regional estimates, except for observed heterozygosity which was consistent with values estimated from South Flank.

Table 3. Genetic diversity estimates from SNP markers of detected individuals in 2022 (this study), 2021-2022 (Prada et al., 2023), 2020-2021 (Ottewell et al., 2022), 2019 (Ottewell et al. 2021), and regional estimates. *N* = Number of individuals; *Na* = Number of alleles; *Ho* = Observed heterozygosity; *uHe* = unbiased expected heterozygosity; *F* = inbreeding coefficient, *Ne* = effective population size.

Pop	N	Na	Ho	uHe	F	Ne (95% CI)
2022 (this study)	11	2.0 ± 0.0	0.35 ± 0.03	0.37 ± 0.02	0.03 ± 0.06	6.4 (2.6-18.1)
2021-2022 (South Flank)	46	2.0 ± 0.0	0.37 ± 0.02	0.45 ± 0.01	0.16 ± 0.03	13.8 (10.2 – 18.7)
2020-2021 (South Flank)	15	2.0 ± 0.0	0.39 ± 0.02	0.46 ± 0.01	0.12 ± 0.04	22.9 (11.1 – 103.5)
2019 (South Flank)	76	2.0 ± 0.0	0.43 ± 0.02	0.47 ± 0.01	0.08 ± 0.04	103.6 (65.1 – 207.9)
East Hamersley	254	2.0 ± 0.0	0.38 ± 0.02	0.47 ± 0.01	0.19 ± 0.04	-

3.6 Relatedness

Relatedness between the 11 individuals detected within the study region was low, with only third-degree relationships or lower (unrelated) (Figure 9).

Pairwise Relatedness Comparisons

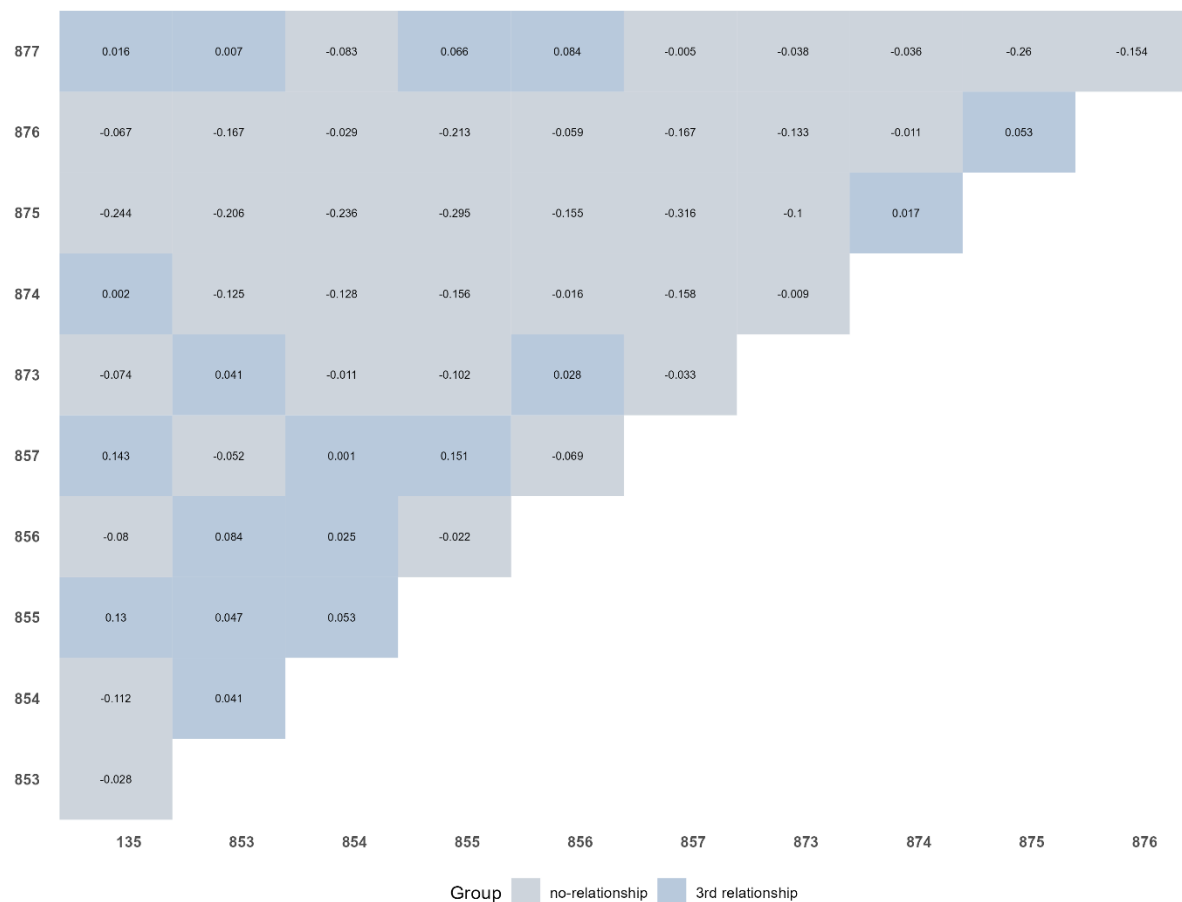


Figure 9. Pairwise relatedness (r) (Ritland 1996) of individuals detected in the Western Ridge-Jimblebar region. First-order relationship ($r = 0.5$; parent – offspring, full-siblings), second-order relationship ($r = 0.25$; half-sibling, uncle/aunt – nephew/niece, grandparent – grandoffspring) and third-order relationship ($r = 0.125$; full cousin, great-grandparent – great-grandoffspring, great-uncle/aunt – great-nephew/niece, half-uncle/aunt – nephew/niece. Values $r < 0.0$ indicates unrelated individuals.

4 Discussion

This report covers genetic analysis of the ghost bat population in the Western Ridge-Jimblebar mining precinct from scats collected in three discontinuous months in 2022 (February, June, and September).

Of the 155 samples collected, 70% (n=108) were confidently genotyped and 11 unique individuals identified. Due to the variable quality of scat samples in this report, we applied moderately conservative filtering thresholds to reach the reported number of individuals. The results therefore represent a minimum number of individuals utilising the caves at Western Ridge-Jimblebar. Both scat collection and the numbers of individuals identified were heavily skewed towards roost CWER-03 (100 scats/9 individuals) indicating the significance of this roost as the centre of ghost bat activity in the area. In addition, female bats were only detected in this roost suggesting it as a putative maternity site. The remaining roosts included in the survey were only used by male bats during the survey period.

Pairwise relatedness values were low for this dataset with no first or second-degree relationships detected. We would have expected to identify close relatedness in the February cohort, as juveniles born in late October-early November of the previous year, may have been roosting with their mothers at this time. Albeit the lack of detection of first-degree relationships (e.g. parent-offspring) may have been due to the small sample size (n = 7 individuals). It is also possible that juveniles were fully weaned by the time the samples were collected.

The presence of scat material in the nine sampled roosts suggest the sites are actively used by ghost bats. Caves CJIM-16 and CJIM-05 appeared to have moderate amounts of scat activity (~20 scats per roost), however, we were only able to successfully genotype a small number of scats from these. Both, CJIM-16 and CJIM-05 are shallow caves, with scats being subject to environmental factors and therefore degradation. In particular, all samples from CJIM-05 were subjected to UV exposure as all the scats were collected from the entrance of the cave, hence explaining the low amplification rate (Chris Knuckey, Biologic Environmental, pers. Comm).. As suggested by the sample rarefaction curve (Figure 3), further sampling effort is expected to detect additional individuals occupying the area.

The patterns of roost use are consistent with those observed elsewhere in the East Hamersley region. The low number of individuals per roost in the study area is consistent with previous observations in the nearby region of South Flank, where roosts appear to be occupied by <10 bats.

Movement patterns are also consistent with regional reports, with bats being recaptured at either the same or nearby roosts (Ottewell et al., 2021, 2022; Prada et al., 2023). We detected movements of 8 and 9 kilometres between roosts in this report, providing further evidence to the hypothesis that ghost bats show fidelity to geographically close (<10 km) roost clusters. Recapture information indicated individual 135 (female) has been present in the area since 2016 and has been alternating between roosts CWER-03 and BHP0B35-02, located 8 km apart. Further,

male 874 was detected moving between roosts CWER-03 and CWER-17 during the survey period (Feb – Sept 2022), located 9 km apart. This use pattern has important management implications as it suggests persistence and viability of ghost bats in the area are linked to the maintenance of groups of interconnected roosts in the landscape.

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Appendices

Appendix 1 Sample details

List of samples included in this report. Collection site, associated dates, and sample identifiers are shown. Sex, genotype, and amplification rate (Amp Rate) are shown per sample. Whether an individual has been previously identified (recapture) is also shown. Refer to text for details.

Roost	Date	Sample	Sex	Genotype	Amp Rate	Recapture
CWER-03	02/26/2022	BMR07540	Female	135	0.86	yes
CWER-03	02/26/2022	BMR07544	Female	135	0.85	yes
CWER-03	02/26/2022	BMR07545	Female	135	0.77	yes
CWER-03	02/26/2022	BMR07548	Female	135	0.94	yes
CWER-03	02/26/2022	BMR07550	Female	135	0.90	yes
CWER-03	02/26/2022	BMR07556	Female	135	0.85	yes
CWER-03	06/11/2022	BMR07648	Female	135	0.92	yes
CWER-03	02/26/2022	BMR07538	Female	853	0.85	no
CWER-03	02/26/2022	BMR07547	Female	854	0.81	no
CWER-03	02/26/2022	BMR07549	Female	855	0.77	no
CWER-03	02/26/2022	BMR07537	Female	856	0.82	yes
CWER-03	02/26/2022	BMR07542	Female	856	0.85	yes
CWER-03	02/26/2022	BMR07543	Female	856	0.85	yes
CWER-03	02/26/2022	BMR07551	Female	856	0.88	yes
CWER-03	02/26/2022	BMR07553	Female	856	0.81	yes
CWER-03	06/11/2022	BMR07620	Female	856	0.96	yes
CWER-03	06/11/2022	BMR07623	Female	856	0.77	yes
CWER-03	06/11/2022	BMR07624	Female	856	0.81	yes
CWER-03	06/11/2022	BMR07627	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07628	Female	856	0.79	yes
CWER-03	06/11/2022	BMR07633	Female	856	0.81	yes
CWER-03	06/11/2022	BMR07635	Female	856	0.85	yes
CWER-03	06/11/2022	BMR07639	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07640	Female	856	0.85	yes
CWER-03	06/11/2022	BMR07641	Female	856	0.92	yes
CWER-03	06/11/2022	BMR07643	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07644	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07646	Female	856	0.82	yes
CWER-03	06/11/2022	BMR07650	Female	856	1.00	yes
CWER-03	06/11/2022	BMR07651	Female	856	0.96	yes

CWER-03	06/11/2022	BMR07654	Female	856	0.88	yes
CWER-03	06/11/2022	BMR07656	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07658	Female	856	0.90	yes
CWER-03	06/11/2022	BMR07660	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07664	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07665	Female	856	0.92	yes
CWER-03	06/11/2022	BMR07668	Female	856	0.92	yes
CWER-03	06/11/2022	BMR07669	Female	856	0.88	yes
CWER-03	06/11/2022	BMR07672	Female	856	0.88	yes
CWER-03	06/11/2022	BMR07674	Female	856	0.92	yes
CWER-03	06/11/2022	BMR07675	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07678	Female	856	0.90	yes
CWER-03	06/11/2022	BMR07682	Female	856	0.96	yes
CWER-03	06/11/2022	BMR07687	Female	856	0.96	yes
CWER-03	06/11/2022	BMR07688	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07690	Female	856	0.96	yes
CWER-03	06/11/2022	BMR07691	Female	856	0.94	yes
CWER-03	06/11/2022	BMR07692	Female	856	0.83	yes
CWER-03	06/11/2022	BMR07693	Female	856	0.88	yes
CWER-03	06/11/2022	BMR07694	Female	856	0.87	yes
CWER-03	02/26/2022	BMR07554	Female	857	0.79	no
CWER-03	06/11/2022	BMR07622	Male	873	0.96	no
CWER-03	06/11/2022	BMR07626	Male	873	0.96	no
CWER-03	06/11/2022	BMR07630	Male	873	0.92	no
CWER-03	06/11/2022	BMR07631	Male	873	0.98	no
CWER-03	06/11/2022	BMR07632	Male	873	0.94	no
CWER-03	06/11/2022	BMR07637	Male	873	0.94	no
CWER-03	06/11/2022	BMR07638	Male	873	0.92	no
CWER-03	06/11/2022	BMR07649	Male	873	0.96	no
CWER-03	06/11/2022	BMR07652	Male	873	0.98	no
CWER-03	06/11/2022	BMR07657	Male	873	0.98	no
CWER-03	06/11/2022	BMR07659	Male	873	0.98	no
CWER-03	06/11/2022	BMR07670	Male	873	0.92	no
CWER-03	06/11/2022	BMR07676	Male	873	0.98	no
CWER-03	06/11/2022	BMR07683	Male	873	0.92	no
CWER-03	06/11/2022	BMR07684	Male	873	0.96	no
CWER-03	06/11/2022	BMR07685	Male	873	0.81	no
CWER-03	06/11/2022	BMR07699	Male	873	0.94	no
CWER-03	06/11/2022	BMR07621	Male	874	0.90	yes
CWER-03	06/11/2022	BMR07625	Male	874	0.98	yes
CWER-03	06/11/2022	BMR07629	Male	874	0.81	yes
CWER-03	06/11/2022	BMR07634	Male	874	0.94	yes
CWER-03	06/11/2022	BMR07636	Male	874	0.98	yes
CWER-03	06/11/2022	BMR07642	Male	874	0.96	yes
CWER-03	06/11/2022	BMR07645	Male	874	1.00	yes
CWER-03	06/11/2022	BMR07647	Male	874	0.94	yes

CWER-03	06/11/2022	BMR07653	Male	874	0.98	yes
CWER-03	06/11/2022	BMR07655	Male	874	0.87	yes
CWER-03	06/11/2022	BMR07661	Male	874	0.94	yes
CWER-03	06/11/2022	BMR07662	Male	874	0.90	yes
CWER-03	06/11/2022	BMR07663	Male	874	0.83	yes
CWER-03	06/11/2022	BMR07666	Male	874	0.96	yes
CWER-03	06/11/2022	BMR07667	Male	874	0.94	yes
CWER-03	06/11/2022	BMR07673	Male	874	0.98	yes
CWER-03	06/11/2022	BMR07677	Male	874	0.91	yes
CWER-03	06/11/2022	BMR07679	Male	874	0.96	yes
CWER-03	06/11/2022	BMR07680	Male	874	0.98	yes
CWER-03	06/11/2022	BMR07681	Male	874	0.96	yes
CWER-03	06/11/2022	BMR07689	Male	874	0.98	yes
CWER-03	06/11/2022	BMR07695	Male	874	0.92	yes
CWER-03	06/11/2022	BMR07696	Male	874	0.88	yes
CWER-03	06/11/2022	BMR07697	Male	874	0.94	yes
CWER-03	06/11/2022	BMR07698	Male	874	0.88	yes
CWER-17	09/07/2022	BMR07730	Male	874	0.78	yes
CNIN-03	06/13/2022	BMR07720	Male	875	0.92	no
CNIN-03	06/13/2022	BMR07723	Male	875	0.94	no
CNIN-03	06/13/2022	BMR07724	Male	875	0.87	no
CJIM-16	09/06/2022	BMR07736	Male	876	0.77	no
CJIM-16	09/06/2022	BMR07737	Male	876	0.79	no
CJIM-16	09/06/2022	BMR07739	Male	876	0.92	no
CJIM-16	09/06/2022	BMR07740	Male	876	0.77	no
CJIM-16	09/06/2022	BMR07741	Male	876	0.81	no
CJIM-16	09/06/2022	BMR07745	Male	876	0.83	no
CJIM-16	09/06/2022	BMR07746	Male	876	0.87	no
CJIM-16	09/06/2022	BMR07747	Male	876	0.78	no
CJIM-16	09/06/2022	BMR07748	Male	876	0.83	no
CJIM-16	09/06/2022	BMR07749	Male	876	0.81	no
CWER-03	02/26/2022	BMR07541	Female	877	0.83	no
CWER-03	02/26/2022	BMR07546	NA	NA	0.58	NA
CWER-20	02/24/2022	BMR07557	NA	NA	0.28	NA
CWER-20	02/24/2022	BMR07558	NA	NA	0.12	NA
CWER-20	02/24/2022	BMR07559	NA	NA	0.29	NA
CWER-03	02/26/2022	BMR07539	Female	NA	0.73	NA
CWER-03	02/26/2022	BMR07552	Female	NA	0.63	NA
CWER-03	02/26/2022	BMR07555	Female	NA	0.35	NA
CWER-01	02/26/2022	BMR07560	Female	NA	0.46	NA
CWER-01	02/26/2022	BMR07561	Female	NA	0.35	NA
CWER-03	06/11/2022	BMR07671	NA	NA	0.54	NA
CWER-03	06/11/2022	BMR07686	NA	NA	0.54	NA
CJIM-05	06/10/2022	BMR07700	NA	NA	0.60	NA
CJIM-05	06/10/2022	BMR07701	NA	NA	0.19	NA
CJIM-05	06/10/2022	BMR07702	NA	NA	0.19	NA

CJIM-05	06/10/2022	BMR07703	NA	NA	0.21	NA
CJIM-05	06/10/2022	BMR07704	NA	NA	0.15	NA
CJIM-05	06/10/2022	BMR07705	NA	NA	0.25	NA
CJIM-05	06/10/2022	BMR07706	NA	NA	0.15	NA
CJIM-05	06/10/2022	BMR07707	NA	NA	0.15	NA
CJIM-05	06/10/2022	BMR07708	NA	NA	0.19	NA
CJIM-05	06/10/2022	BMR07709	NA	NA	0.17	NA
CJIM-05	06/10/2022	BMR07710	NA	NA	0.25	NA
CJIM-05	06/10/2022	BMR07711	NA	NA	0.21	NA
CJIM-05	06/10/2022	BMR07712	NA	NA	0.27	NA
CJIM-05	06/10/2022	BMR07713	NA	NA	0.15	NA
CJIM-05	06/10/2022	BMR07714	NA	NA	0.19	NA
CJIM-05	06/10/2022	BMR07715	NA	NA	0.33	NA
CJIM-05	06/10/2022	BMR07716	NA	NA	0.29	NA
CJIM-05	06/10/2022	BMR07717	NA	NA	0.25	NA
CJIM-05	06/10/2022	BMR07718	NA	NA	0.29	NA
CJIM-05	06/10/2022	BMR07719	NA	NA	0.29	NA
CNIN-03	06/13/2022	BMR07721	NA	NA	0.75	NA
CNIN-03	06/13/2022	BMR07722	NA	NA	0.75	NA
CJIM-15	09/06/2022	BMR07725	NA	NA	0.35	NA
CJIM-15	09/06/2022	BMR07726	NA	NA	0.19	NA
CNIN-01	09/06/2022	BMR07727	NA	NA	0.23	NA
CNIN-01	09/06/2022	BMR07728	NA	NA	0.06	NA
CWER-17	09/07/2022	BMR07729	NA	NA	0.75	NA
CJIM-16	09/06/2022	BMR07731	NA	NA	0.75	NA
CJIM-16	09/06/2022	BMR07732	NA	NA	0.73	NA
CJIM-16	09/06/2022	BMR07733	NA	NA	0.75	NA
CJIM-16	09/06/2022	BMR07734	NA	NA	0.73	NA
CJIM-16	09/06/2022	BMR07735	NA	NA	0.73	NA
CJIM-16	09/06/2022	BMR07738	NA	NA	0.67	NA
CJIM-16	09/06/2022	BMR07742	NA	NA	0.63	NA
CJIM-16	09/06/2022	BMR07743	NA	NA	0.75	NA
CJIM-16	09/06/2022	BMR07744	NA	NA	0.65	NA

Appendix 2 Sampling and Genotyping Summary

Roosts, associated number of samples analysed, successfully amplified, and the number of unique genotypes detected from each site.

Roost	Samples Analysed	Successfully Amplified	Unique Genotypes
CJIM-15	2	0	0
CNIN-01	2	0	0
CJIM-05	20	0	0
CJIM-16	19	10	1
CNIN-03	5	3	1
CWER-01	2	0	0
CWER-03	100	94	9
CWER-17	2	1	1
CWER-20	3	0	0

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Appendix H: Diet Analysis Results

Appendix Table 8: Diet analysis

Sample	Budgerigar	Bird	<i>Mus musculus</i>	<i>Dasyurid</i>	Beetle	Moth/ butterfly	Unidentified Insect	Seed	Total	Comments
1		40	40			20			3	
2	70		25	5					3	
3		20	60			20			3	
4	80							20	2	
5	10		90						2	
6	95		5						2	
7	95		5						2	
8	80		20						2	
9	99		1						2	
10		90					10		2	
11		1	99						2	
12	20				80				2	
13	50		50						2	
14	10		90						2	
15			20			80			2	
16	100								1	
17			100						1	
18	100								1	
19		100							1	
20	100								1	
21	100								1	
22	100								1	
23	100								1	
24		100							1	
25	100								1	
26	100								1	
27		100							1	
28		100							1	
29		100							1	red/brown feather

Sample	Budgerigar	Bird	<i>Mus musculus</i>	<i>Dasyurid</i>	Beetle	Moth/ butterfly	Unidentified Insect	Seed	Total	Comments
30			100						1	
31			100						1	
32		100							1	red/brown feather
33	100								1	
34		100							1	
35	100								1	
36	100								1	
37	100								1	
38	100								1	
39			100						1	
40	100								1	
41		100							1	
42	100								1	
43	100								1	
44	100								1	
45	100								1	
46		100							1	red/brown feather
47			100						1	
48			100						1	
49	100								1	
50	100								1	
51	100								1	
52	100								1	
53	100								1	
54	100								1	
55	100								1	
56			100						1	
57	100								1	
58	100								1	
59			100						1	
60	100								1	

Sample	Budgerigar	Bird	<i>Mus musculus</i>	<i>Dasyurid</i>	Beetle	Moth/ butterfly	Unidentified Insect	Seed	Total	Comments
61	100								1	
62	100								1	
63		100							1	
64			100						1	
65			100						1	
66			100						1	
67		100							1	
68		100							1	
69	100								1	
70					100				1	
71	100								1	
72		100							1	
73			100						1	
74			100						1	
75			100						1	
76	100								1	
77	100								1	
78			100						1	
79					100				1	
80	100								1	
81			100						1	
82					100				1	
83	100								1	
84	100								1	
85	100								1	
86			100						1	red/brown feather
87			100						1	red/brown feather
88		100							1	
89	100								1	
90	100								1	
91			100						1	

Sample	Budgerigar	Bird	<i>Mus musculus</i>	<i>Dasyurid</i>	Beetle	Moth/ butterfly	Unidentified Insect	Seed	Total	Comments
92		100							1	
93	100								1	
94	100								1	
95		100							1	
96					100				1	
97	100								1	
98	100								1	
99		100							1	
100			100						1	
101			100						1	
102		100							1	
103	100								1	
104			100						1	
105		100							1	
106			100						1	
107			100						1	
108		100							1	
109	100								1	
110			100						1	
111		100							1	red/brown feather
112		100							1	red/brown feather
113			100						1	
114	100								1	
115	100								1	
116	100								1	
117	100								1	
118	100								1	
119	100								1	
120			100						1	
121					100				1	
122	100								1	

Sample	Budgerigar	Bird	<i>Mus musculus</i>	<i>Dasyurid</i>	Beetle	Moth/ butterfly	Unidentified Insect	Seed	Total	Comments
123			100						1	
124		100							1	
125		100							1	
126		100							1	
127	100								1	
128					100				1	
129	100								1	
130		100							1	
131			100						1	
132	100								1	
133		100							1	
134		100							1	
135	100								1	
136		100							1	
137	100								1	
138			100						1	
139			100						1	
140		100							1	
141		100							1	
142		100							1	
143	100								1	
144					100				1	
145			100						1	
146			100						1	
147	100								1	
Total	70	36	45	1	8	3	1	1		

Note – table recreated/ formatted from the results received from Scats About

Appendix I: Camera Monitoring Results

Appendix Table 9: Motion camera results

Area	Cave ID	Date	Civil Twilight (Dusk)	Civil Twilight (Dawn)	Time First Seen	Time Last Seen	Max observed
Western Ridge	CWER-01	14/05/23	17:52	6:03	3:37	Flyby	1
					3:38		
	CWER-01	25/06/23	17:49	6:18	3:18	3:21	1
	CWER-01	26/06/23	17:50	6:18	20:32	20:36	1
	CWER-01	27/06/23	17:50	6:18	22:48	Flyby	1
					20:57	23:38	
					23:39	Flyby	
	CWER-03	12/04/23	18:14	5:51	3:22	3:25	1
					3:58	4:00	
	CWER-03	29/07/23	18:01	6:14	4:40	Flyby	1
					4:44		
	CWER-17	15/05/23	18:31	5:53	3:05	3:07	1
Jimblebar / Ninga	CJIM-05	26/06/23	17:50	6:18	2:04	Flyby	1
	CJIM-05	29/06/23	17:50	6:19	2:55	2:56	1
					3:01	3:02	
	CJIM-05	30/06/23	17:51	6:19	0:00	Flyby	1
					0:35		
					0:31		
					2:31		
					3:16		
					3:19		
					3:20		
					3:29		
	CNIN-03	16/04/23	18:10	5:52	1:57	2:01	1
	CNIN-03	20/04/23	18:07	5:53	3:18	3:22	1
	CNIN-03	16/05/23	17:51	6:04	3:44	Flyby	1
	CNIN-03	20/05/23	17:50	6:06	1:40	Flyby	1
					1:41		
					3:51		
					4:02		
	CNIN-03	21/05/23	17:49	6:06	4:46	Flyby	1

Area	Cave ID	Date	Civil Twilight (Dusk)	Civil Twilight (Dawn)	Time First Seen	Time Last Seen	Max observed
	CNIN-03	25/05/23	17:48	6:08	1:39	5:52	1
	CNIN-03	29/05/23	17:47	6:09	1:35	Flyby	1
	CNIN-03	20/07/23	17:58	6:17	1:53	Flyby	1
					2:16		
					2:56		
	CNIN-09	19/04/23	18:08	5:53	3:17	3:47	1
	CNIN-09	14/05/23	17:52	6:03	3:01	4:01	1
	CNIN-09	24/05/23	17:48	6:07	5:11	5:15	1
	CNIN-09	29/05/23	17:47	6:09	5:21	Flyby	2
	CNIN-09	1/07/23	17:51	6:19	4:27	4:33	1
	CNIN-09	2/07/23	17:51	6:19	0:45	12:48	1
					3:29	3:33	
					4:30	4:31	
					4:35	Flyby	
					5:00		

Appendix J: Microclimate Results

Appendix Table 10: Summary of internal temperature data recorded at the roosts

Area	Cave ID	Category	Mean ($\pm$ standard error)	Min °C	Max °C	Difference between Min and Max °C	% Recordings within Preferred Range
Western Ridge	CWER-01	Category 2	27.81$\pm$0.04	24.07	33.11	9.04	43.48
	CWER-03	Category 2	29.56$\pm$0.03	26.75	33.98	7.22	49.32
	CWER-04	Category 4	27.82 $\pm$ 0.06	23.03	33.44	10.41	31.20
	CWER-05	Category 4	27.02 $\pm$ 0.08	19.96	34.37	14.40	14.45
	CWER-07	Category 4	27.76 $\pm$ 0.06	22.63	33.95	11.31	31.19
	CWER-09	Category 4	28.45 $\pm$ 0.01	27.15	29.69	2.54	63.43
	CWER-10	Category 3	26.12 $\pm$ 0.06	21.29	32.52	11.22	36.58
	CWER-14	Category 4	25.51 $\pm$ 0.1	14.90	35.48	20.58	16.81
	CWER-17	Category 3	29.35$\pm$0.05	25.49	33.77	8.27	33.98
	CWER-20	Category 4	27.88 $\pm$ 0.06	22.09	34.28	12.19	28.77
Jimblebar/ Ninga	CJIM-01	Category 4	28.31 $\pm$ 0.07	21.97	36.75	14.8	20.43
	CJIM-03	Category 2	29.42 $\pm$ 0.05	25.37	34.55	9.18	33.26
	CJIM-04	Category 4	31.4 $\pm$ 0.07	24.51	37.57	13.06	33.83
	CJIM-05*	Category 4	27.69$\pm$0.08	16.92	37.56	20.64	20.02
	CJIM-06	Category 4	25.63 $\pm$ 0.09	15.91	35.22	19.31	23.39
	CJIM-07	Category 4	25.6 $\pm$ 0.1	16.59	35.67	19.07	12.20
	CJIM-08	Category 4	26.88 $\pm$ 0.04	23.20	31.45	8.24	40.48
	CJIM-09*	Category 3	25.88 $\pm$ 0.06	23.52	30.42	6.89	23.67
	CJIM-14	Category 3	30.22 $\pm$ 0.03	27.96	33.36	5.39	77.80
	CJIM-15	Category 4	27.96 $\pm$ 0.06	21.20	34.25	13.05	29.29
	CJIM-16	Category 3	26.56 $\pm$ 0.05	19.19	33.16	13.97	34.51
	CJIM-17	Category 4	27.57 $\pm$ 0.07	19.49	35.51	16.02	17.94
	CJIM-18	Category 4	26.21 $\pm$ 0.09	18.31	36.20	17.88	15.58
	CJIM-20	Category 4	28.93 $\pm$ 0.08	18.32	38.10	19.77	21.37
	CNIN-01	Category 3	29.4$\pm$0.04	26.00	32.45	6.44	69.67
	CNIN-02	Category 3	28.17 $\pm$ 0.02	26.44	30.16	3.72	51.37
	CNIN-03*	Category 2	28.28$\pm$0.01	27.62	29.43	1.81	64.86
	CNIN-13	Category 3	27.27$\pm$0.07	20.43	34.30	13.86	19.42
	CCAT-01	Category 3	29.14 $\pm$ 0.02	27.60	31.88	4.27	71.44
Cathedral Gorge	CCAT-02	Category 3	30.8 $\pm$ 0.02	29.45	33.85	4.39	80.86
	CCAT-06	Category 3	29.93 $\pm$ 0.02	28.04	31.96	3.92	100.00
	CCAT-09	Category 3	29.46 $\pm$ 0.02	27.85	32.65	4.80	93.82
	CCAT-10*	Category 4	-	-	-	-	-

Area	Cave ID	Category	Mean ($\pm$ standard error)	Min °C	Max °C	Difference between Min and Max °C	% Recordings within Preferred Range
	CCAT-13*	Category 4	28.66 $\pm$ 0.05	26.57	31.88	5.30	56.36
	CCAT-14	Category 4	29.07 $\pm$ 0.07	20.67	35.05	14.38	24.11
	CCAT-17	Category 4	30 $\pm$ 0.05	25.95	35.79	9.84	33.22
	CCAT-21*	Category 3	30.24 $\pm$ 0.01	28.95	32.09	3.14	99.94
	CHST-08	Category 4	29.02 $\pm$ 0.07	22.97	36.41	13.44	21.38

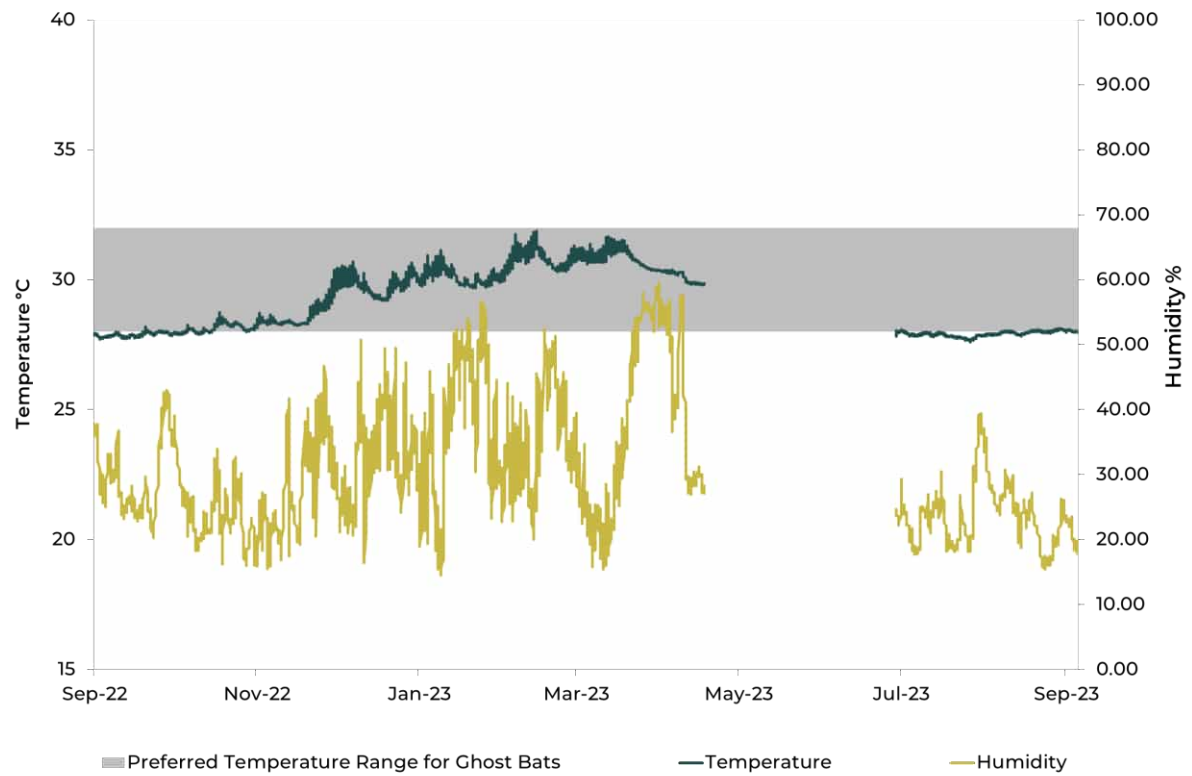
Note: **bolded** refers to caves where ghost bats were recorded via primary or secondary evidence. Asterix refers to caves where available data is not representative of the entire monitoring period and thus means results should be interpreted accordingly.

Appendix Table 11: Summary of internal relative humidity data recorded at the roosts

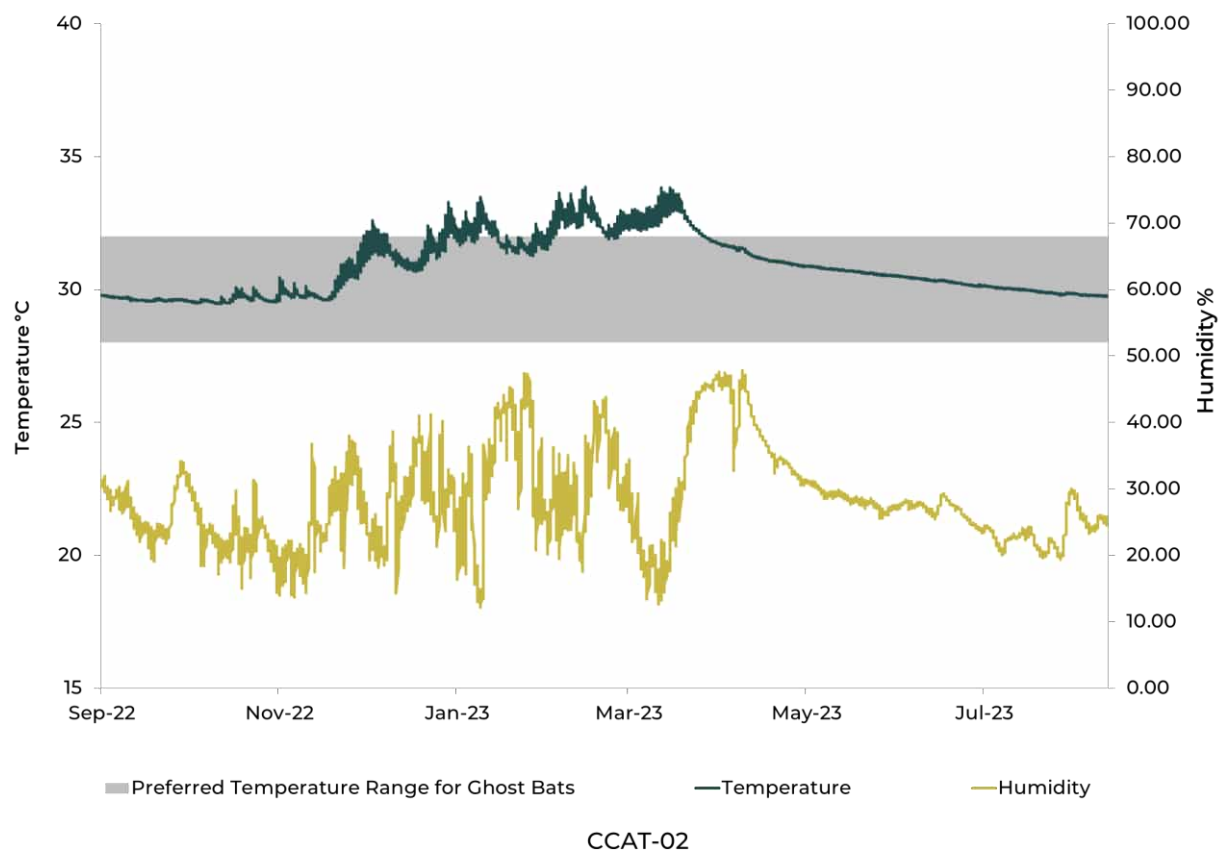
Area	Cave ID	Category	Mean ($\pm$ standard error) (%)	Minimum (%)	Maximum (%)	Difference between Minimum and Maximum
Western Ridge	CWER-01	Category 2	31.39$\pm$0.13	12.86	52.89	40.02
	CWER-03	Category 2	27.63$\pm$0.12	10.75	45.80	35.04
	CWER-04	Category 4	30.36 $\pm$ 0.13	12.43	56.43	43.99
	CWER-05	Category 4	30.57 $\pm$ 0.14	12.22	54.83	42.61
	CWER-07	Category 4	27.84 $\pm$ 0.13	9.97	49.27	39.30
	CWER-09	Category 4	36.97 $\pm$ 0.13	18.88	51.77	32.88
	CWER-10	Category 3	32.64 $\pm$ 0.15	11.78	59.20	47.42
	CWER-14	Category 4	31.68 $\pm$ 0.19	10.62	67.54	56.92
	CWER-17	Category 3	40.76$\pm$0.13	17.63	48.17	30.53
	CWER-20	Category 4	25.62 $\pm$ 0.12	10.21	45.65	35.43
Jimblebar/ Ninga	CJIM-01	Category 4	30.69 $\pm$ 0.16	13.07	59.20	46.13
	CJIM-03	Category 2	25.57 $\pm$ 0.14	8.95	48.80	39.85
	CJIM-04	Category 4	26.93 $\pm$ 0.17	9.31	56.86	47.55
	CJIM-05*	Category 4	27.06$\pm$0.18	8.77	62.73	53.95
	CJIM-06	Category 4	32.82 $\pm$ 0.2	10.62	63.79	53.16
	CJIM-07	Category 4	29.06 $\pm$ 0.17	9.57	58.79	49.21
	CJIM-08	Category 4	33.94 $\pm$ 0.13	15.26	56.99	41.73
	CJIM-09	Category 3	61.77 $\pm$ 0.02	58.38	62.89	4.50
	CJIM-14	Category 3	27.8 $\pm$ 0.16	12.94	53.91	40.96
	CJIM-15	Category 4	28.48 $\pm$ 0.18	10.21	60.75	50.53
	CJIM-16	Category 3	29.06 $\pm$ 0.2	9.70	64.40	54.69
	CJIM-17	Category 4	29.34 $\pm$ 0.18	9.23	60.74	51.50
	CJIM-18	Category 4	28.3 $\pm$ 0.16	9.95	54.96	45.01
	CJIM-20	Category 4	27.97 $\pm$ 0.19	7.95	60.03	52.08
	CNIN-01	Category 3	34.08$\pm$0.19	16.16	60.64	44.48
	CNIN-02	Category 3	32.24 $\pm$ 0.17	18.00	54.26	36.25
	CNIN-03*	Category 2	31.33$\pm$0.12	20.86	50.00	29.14
	CNIN-13	Category 3	38.59$\pm$0.16	13.48	60.38	46.90
Cathedral Gorge	CCAT-01	Category 3	30.67 $\pm$ 0.2	14.53	59.49	44.96
	CCAT-02	Category 3	27.93 $\pm$ 0.13	12.19	47.86	35.66
	CCAT-06	Category 3	55.9 $\pm$ 0.25	19.79	75.50	55.71
	CCAT-09	Category 3	32.11 $\pm$ 0.12	12.28	50.87	38.59
	CCAT-10*	Category 4	-	-	-	-

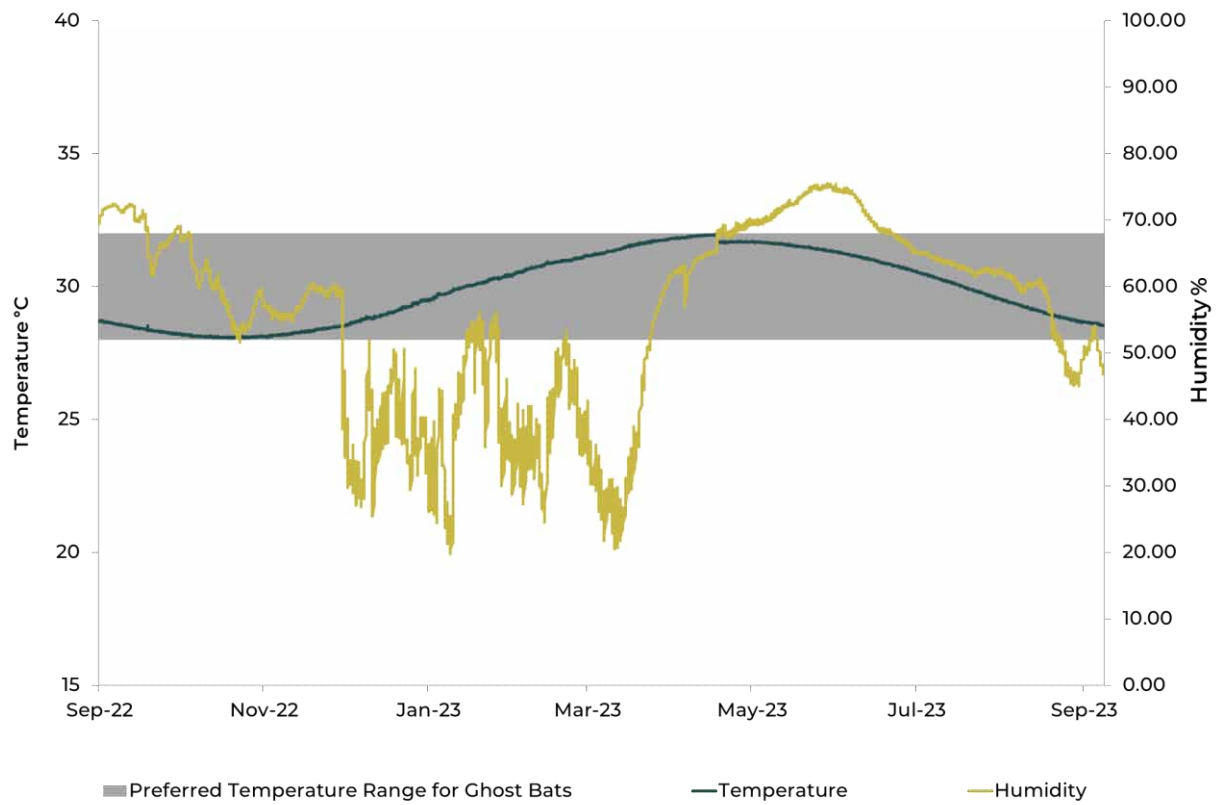
Area	Cave ID	Category	Mean ($\pm$ standard error) (%)	Minimum (%)	Maximum (%)	Difference between Minimum and Maximum
	CCAT-13*	Category 4	47.54 $\pm$ 0.38	29.85	70.16	40.31
	CCAT-14	Category 4	28.87 $\pm$ 0.17	8.03	52.36	44.33
	CCAT-17	Category 4	24.26 $\pm$ 0.11	10.69	42.23	31.54
	CCAT-21*	Category 3	31.09 $\pm$ 0.23	13.16	56.28	43.12
	CHST-08	Category 4	27.47 $\pm$ 0.15	11.02	52.08	41.06

Note: **bolded** refers to caves where ghost bats were recorded via primary or secondary evidence. Asterix refers to caves where available data is not representative of the entire monitoring period and thus means results should be interpreted accordingly.

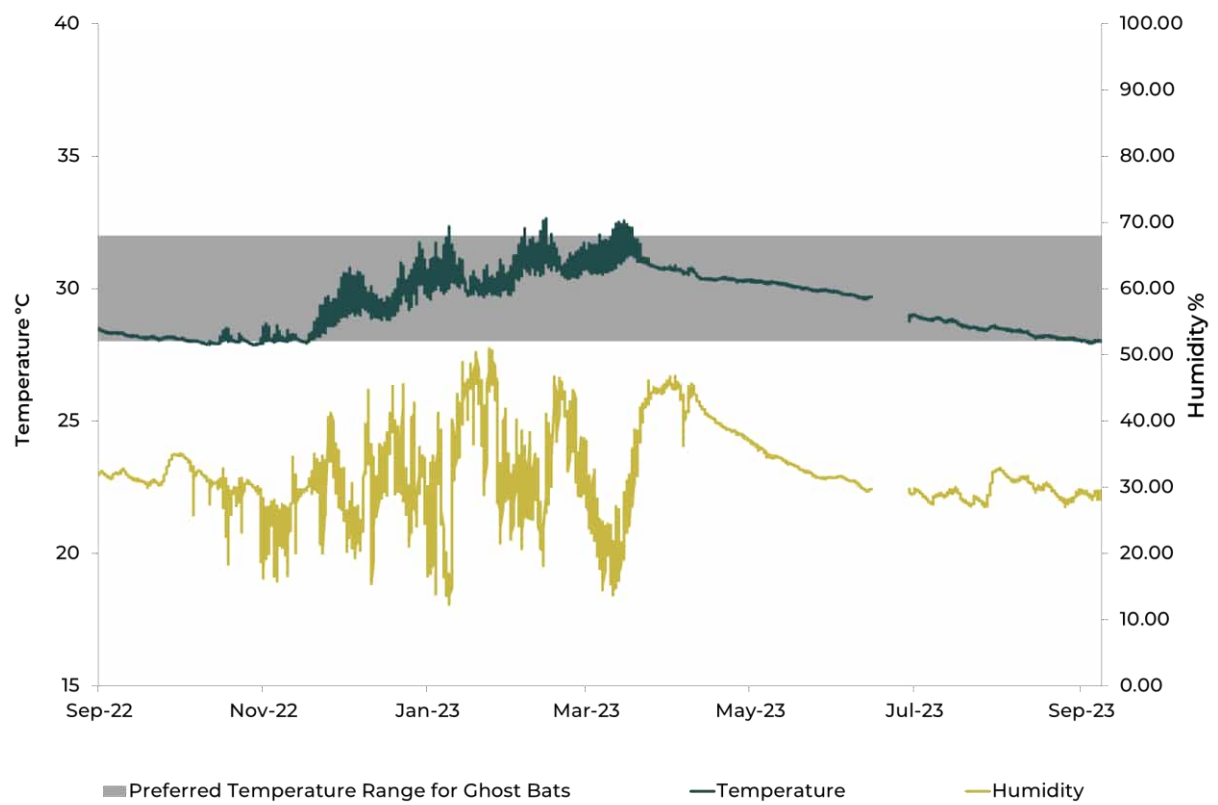


CCAT-01

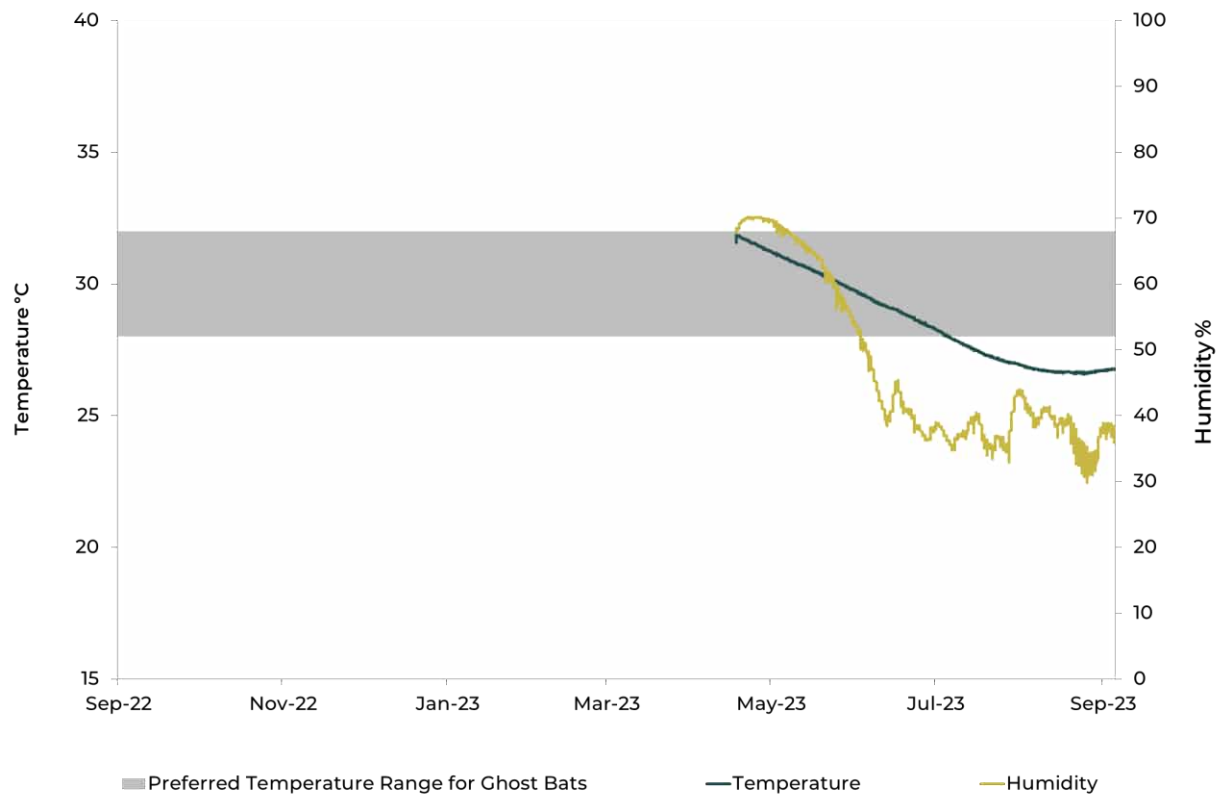




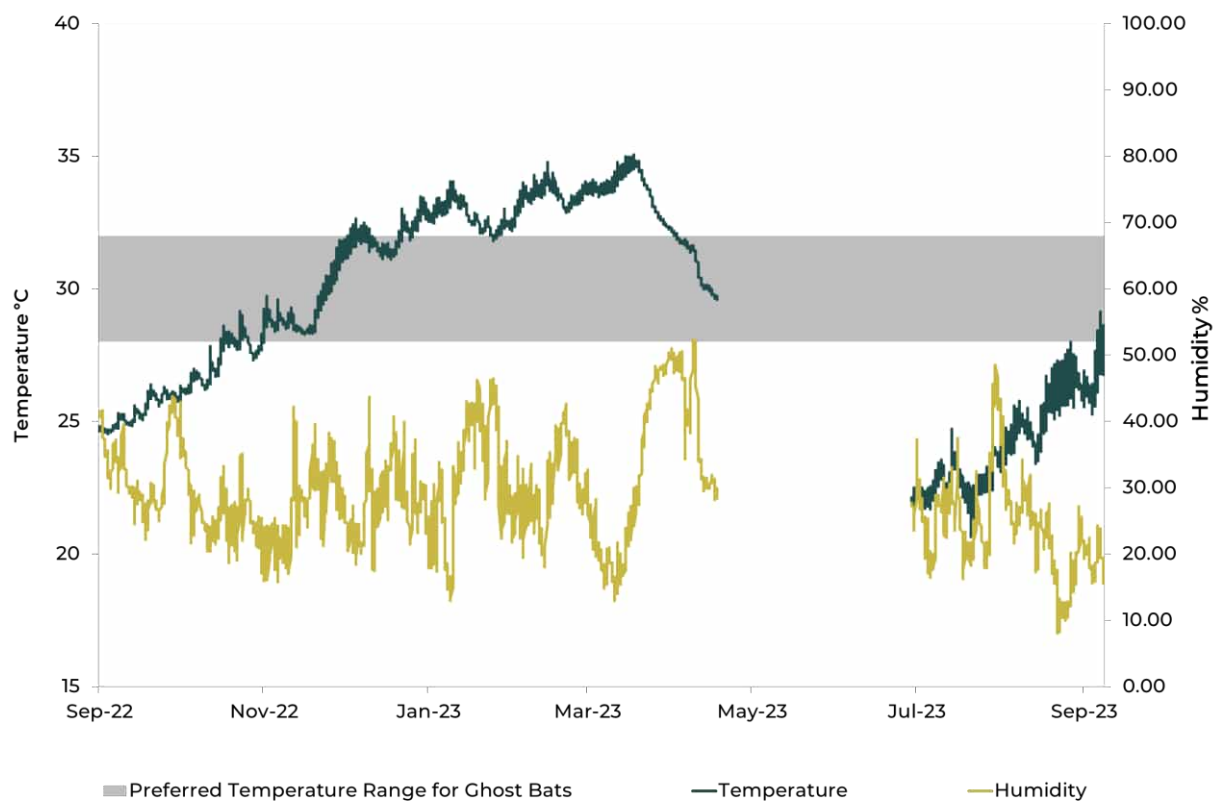
CCAT-06



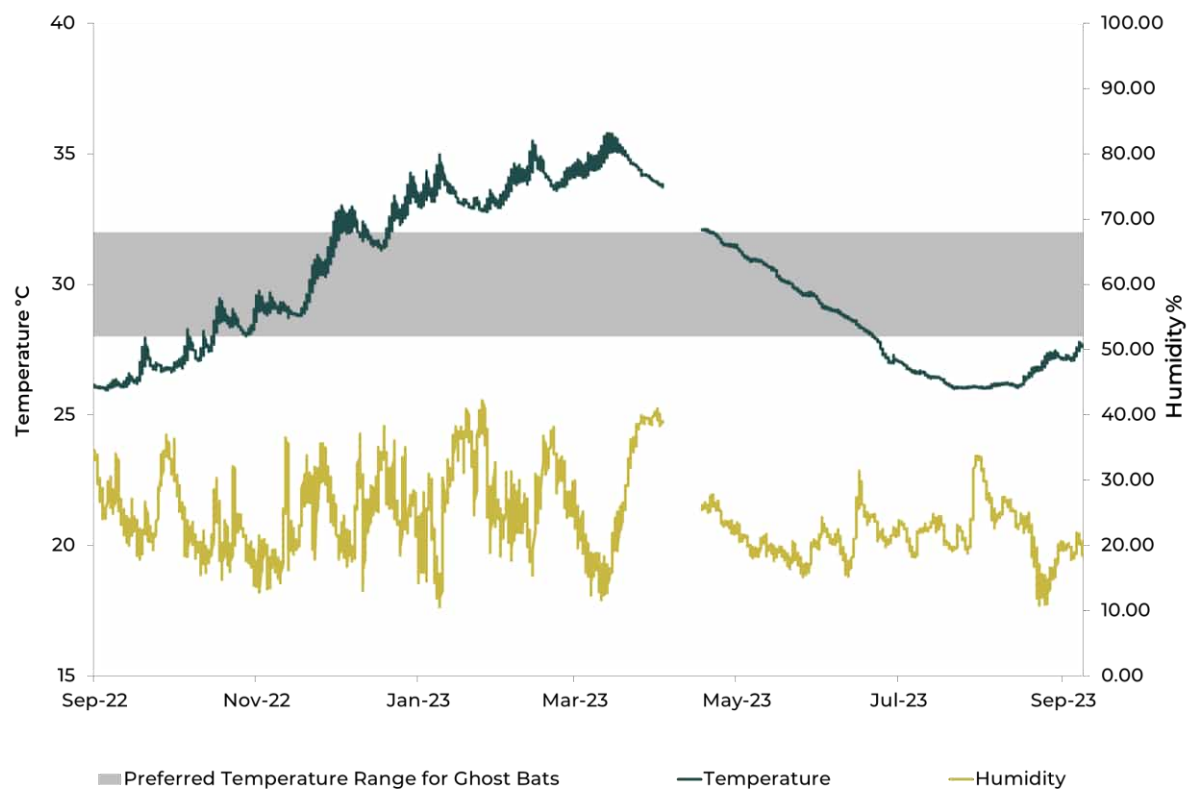
CCAT-09



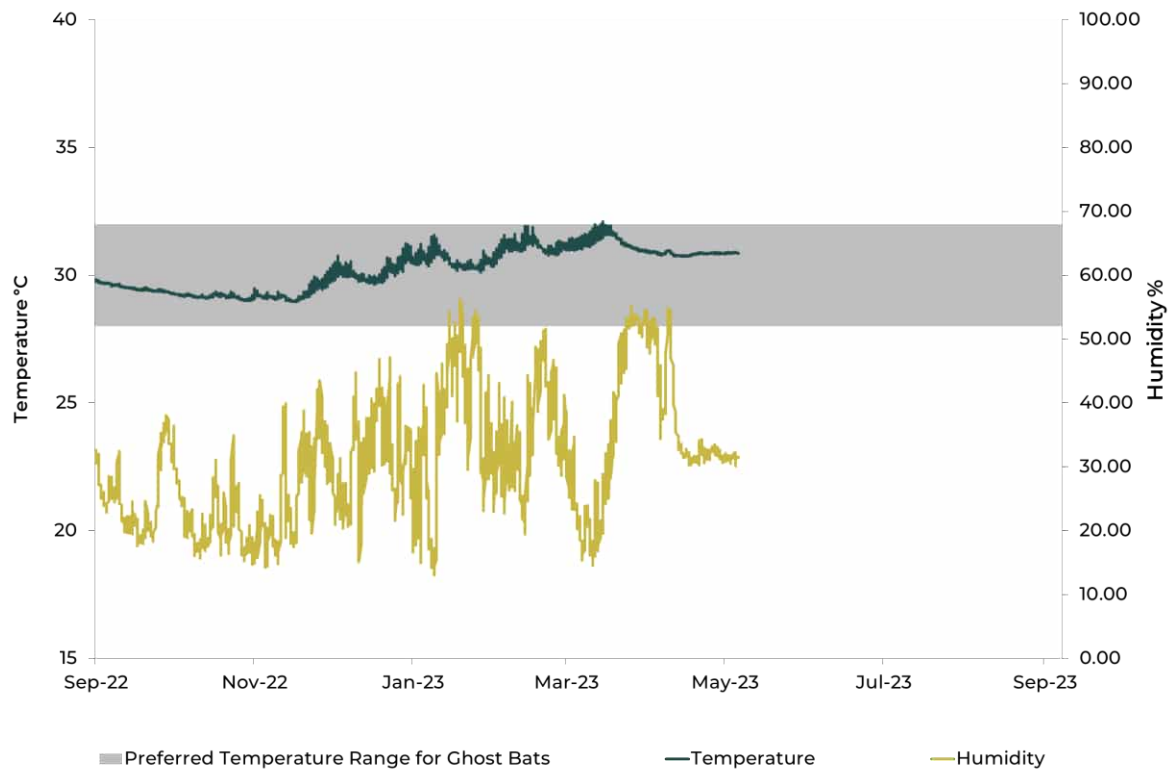
CCAT-13



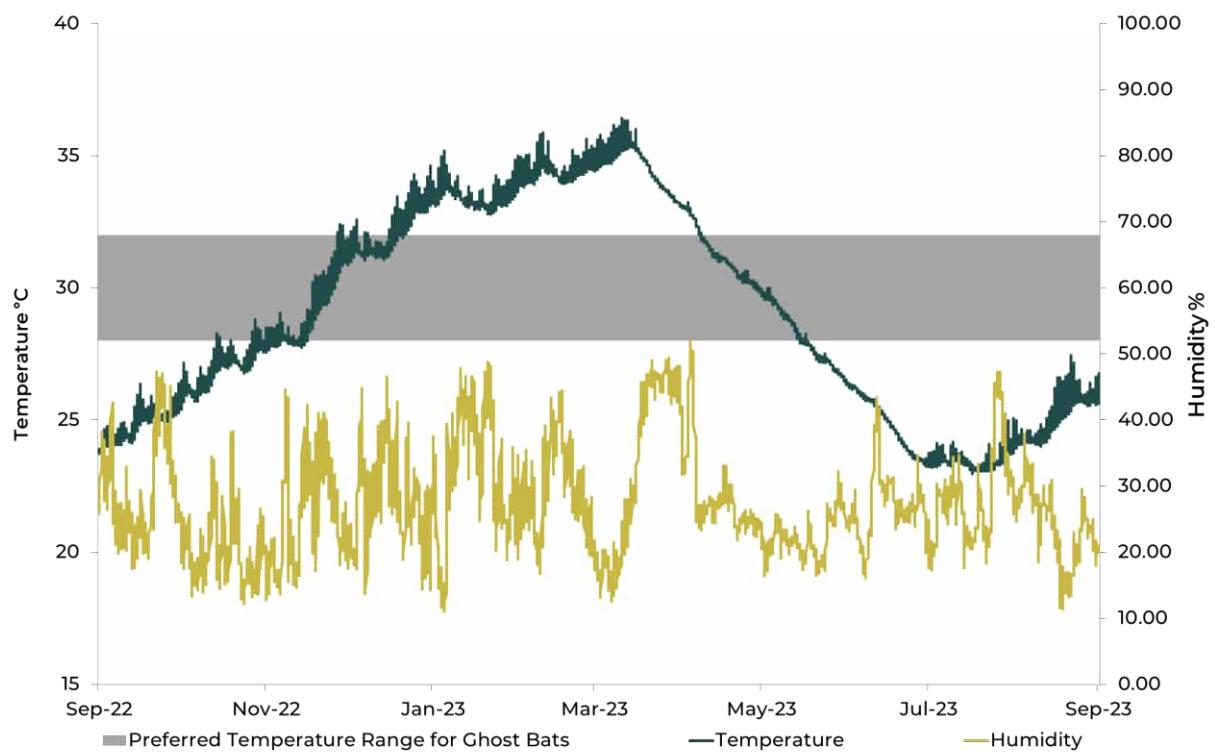
CCAT-14



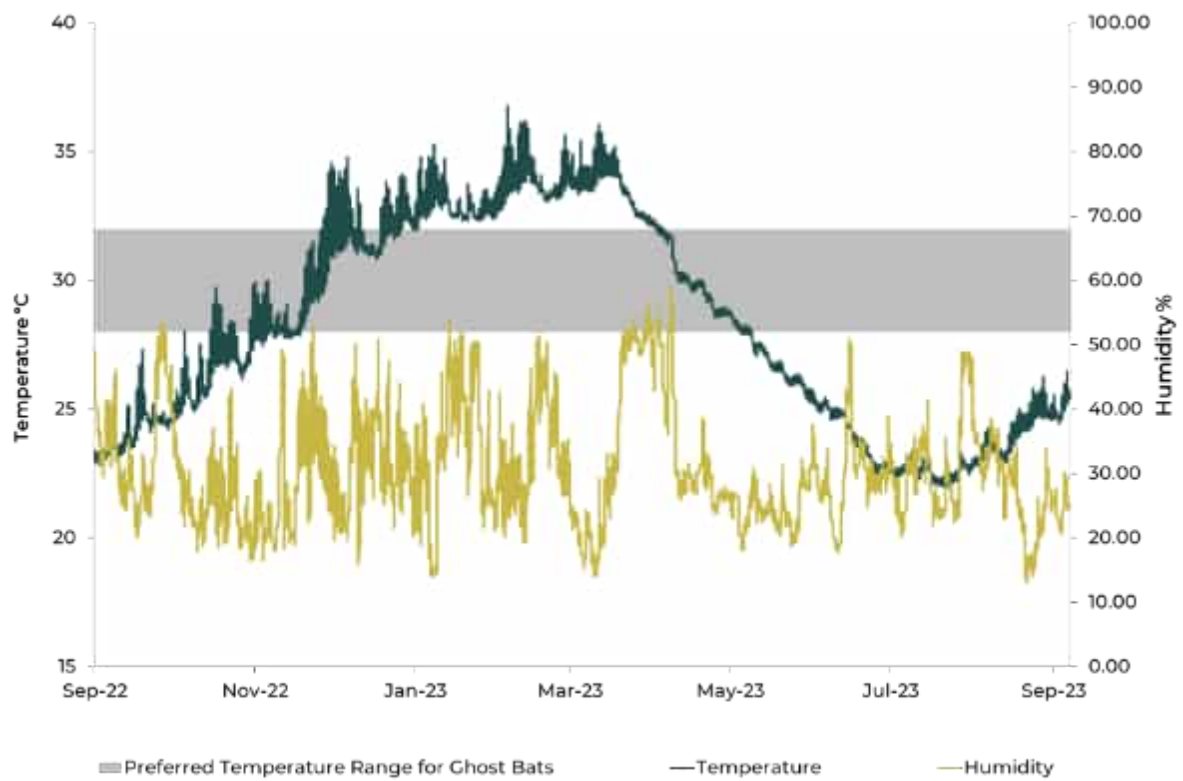
CCAT-17



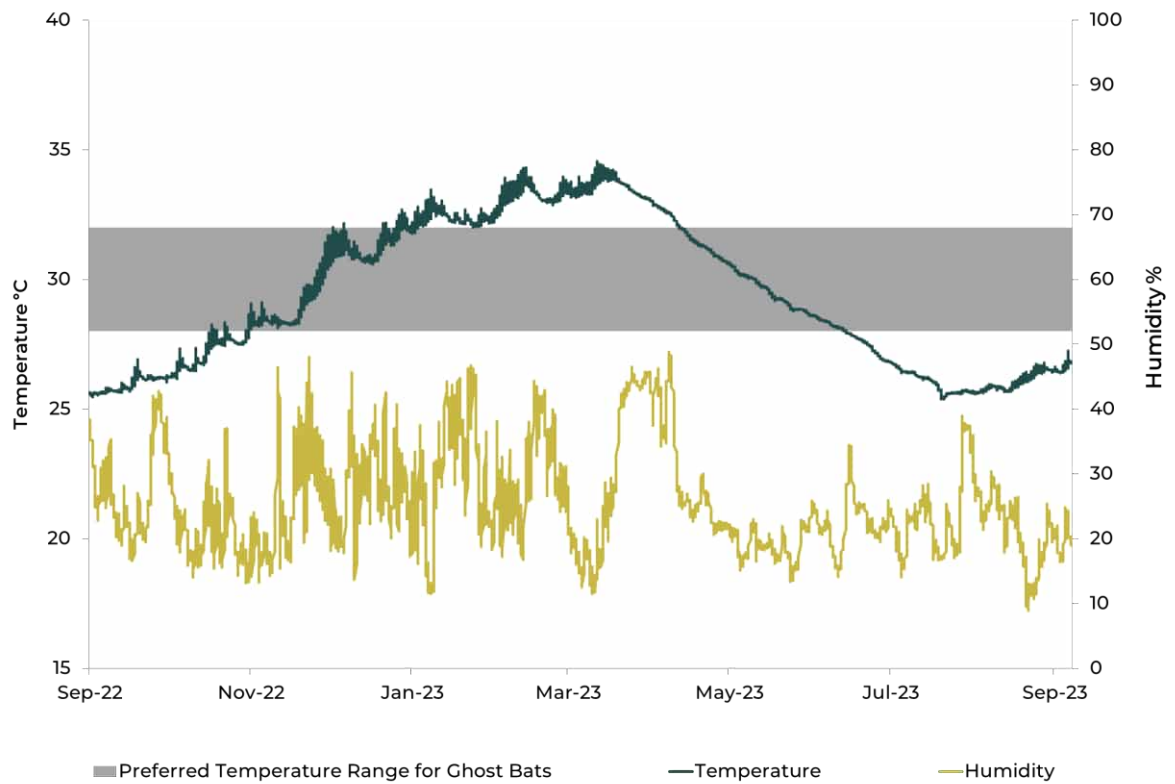
CCAT-21



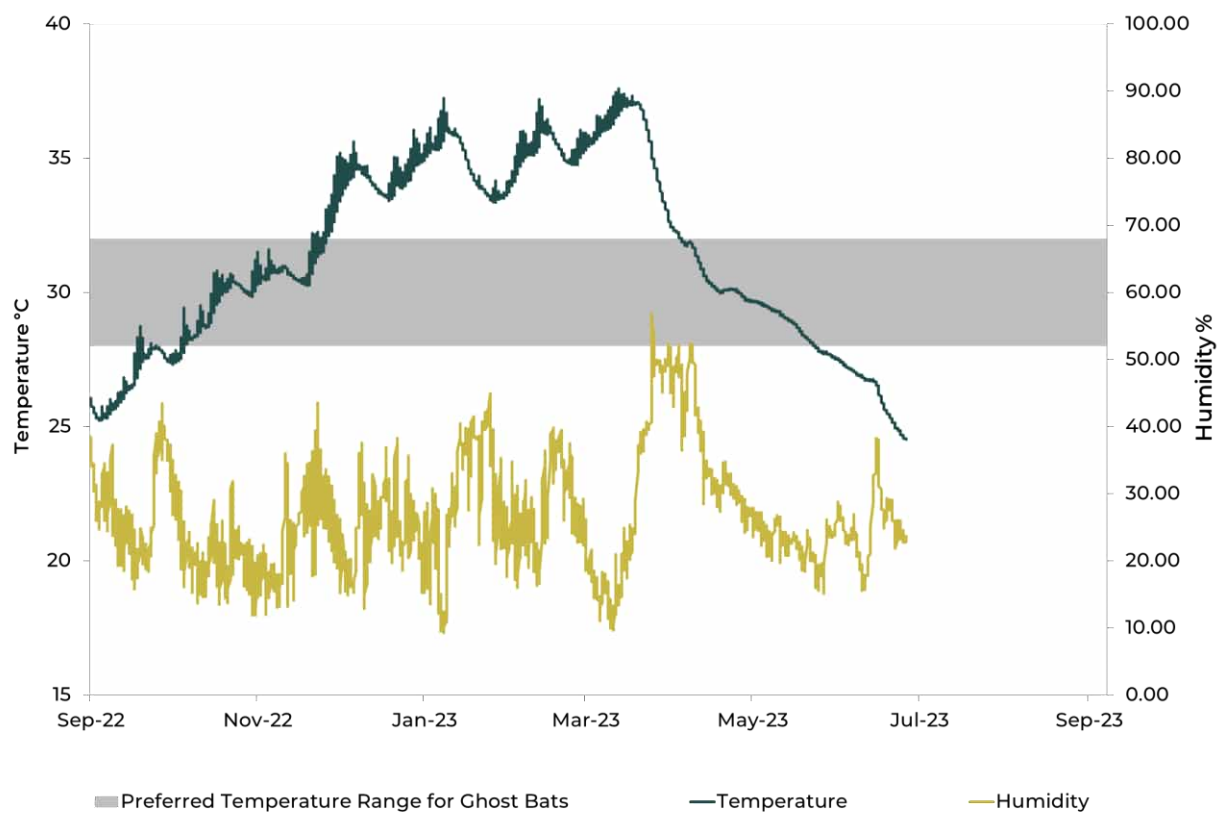
CHST-08



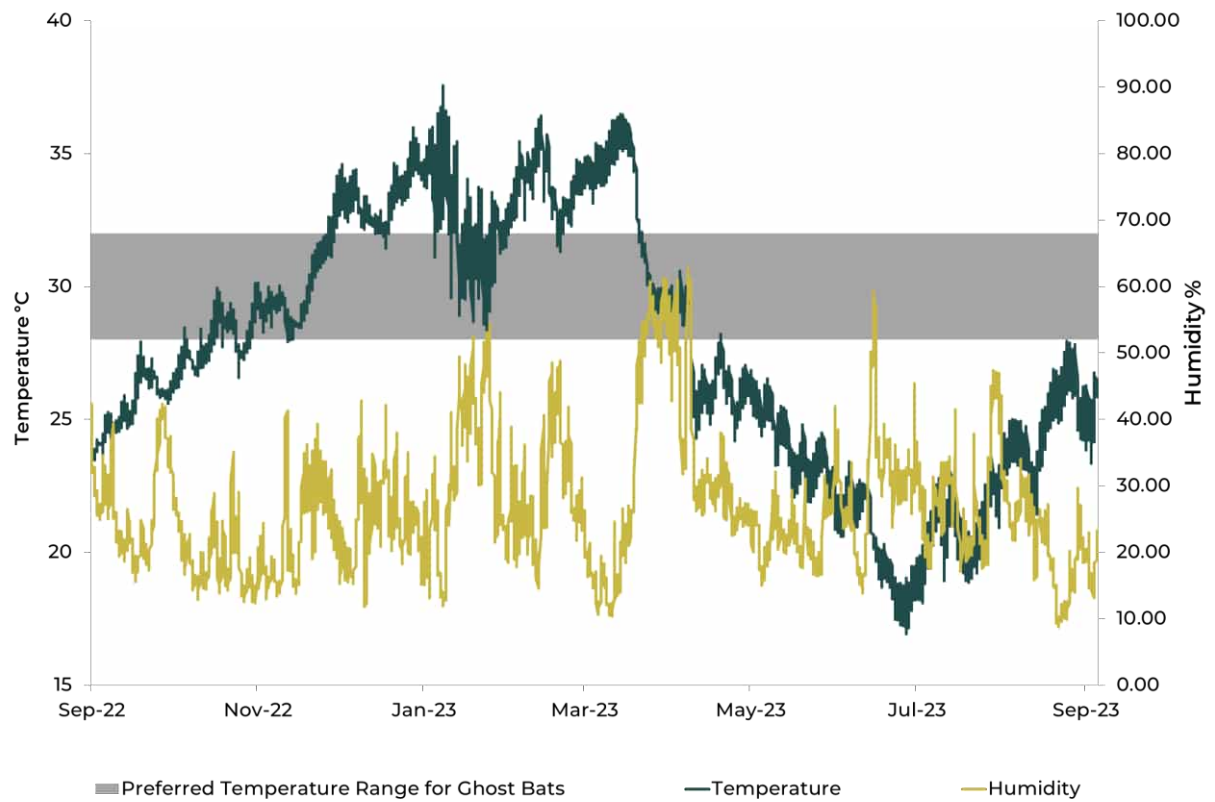
CJIM-01



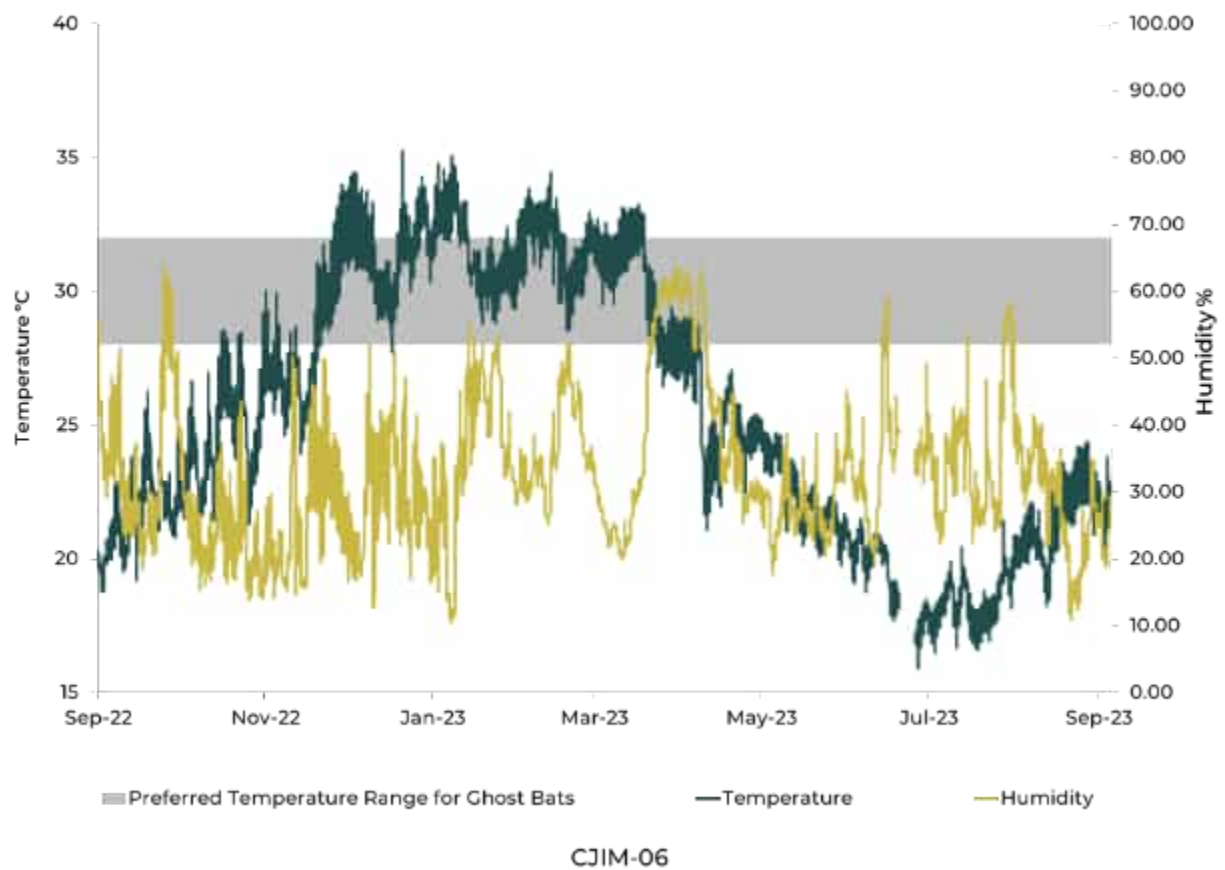
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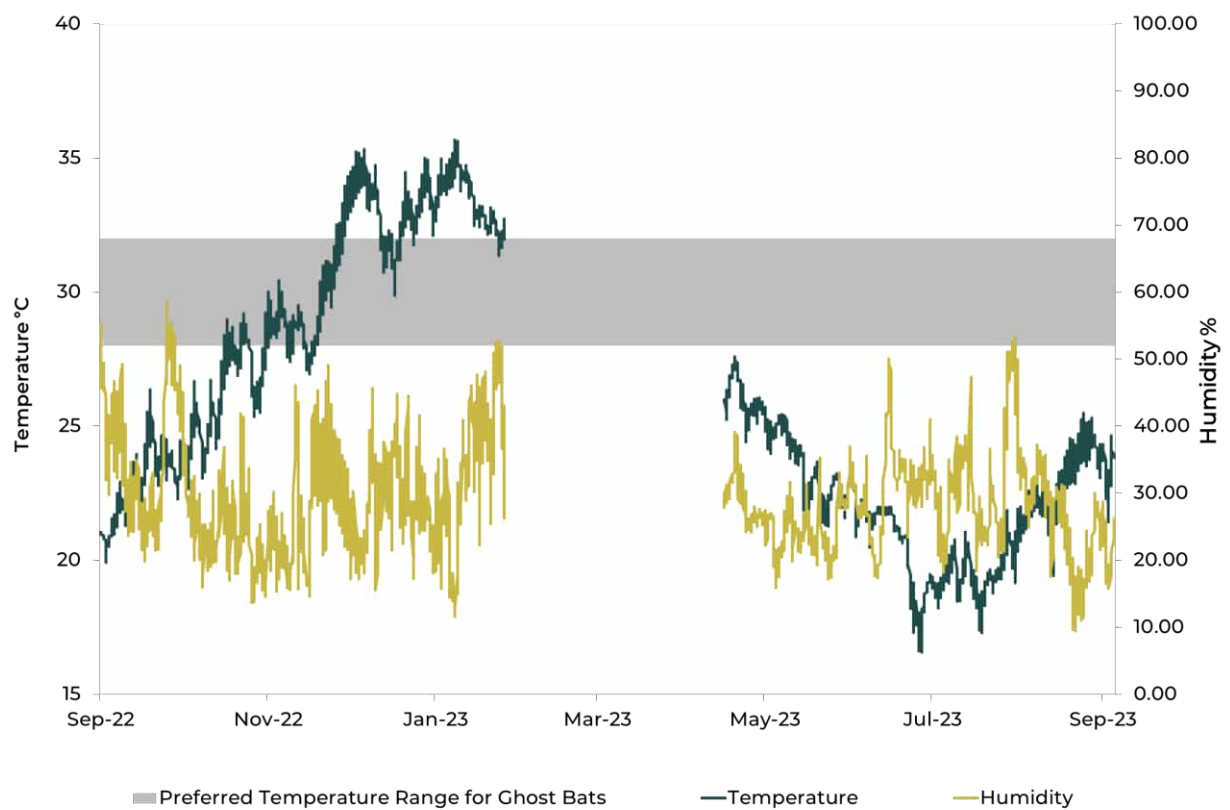


CJIM-04

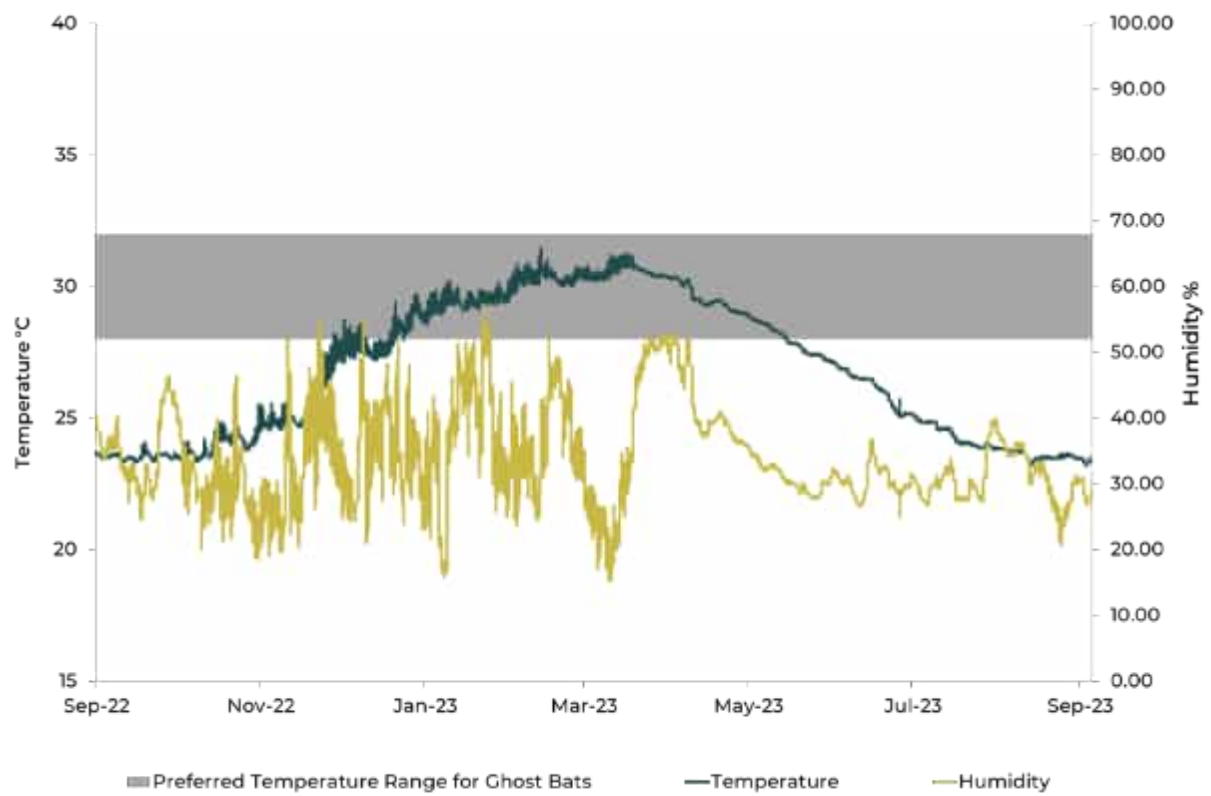


CJIM-05

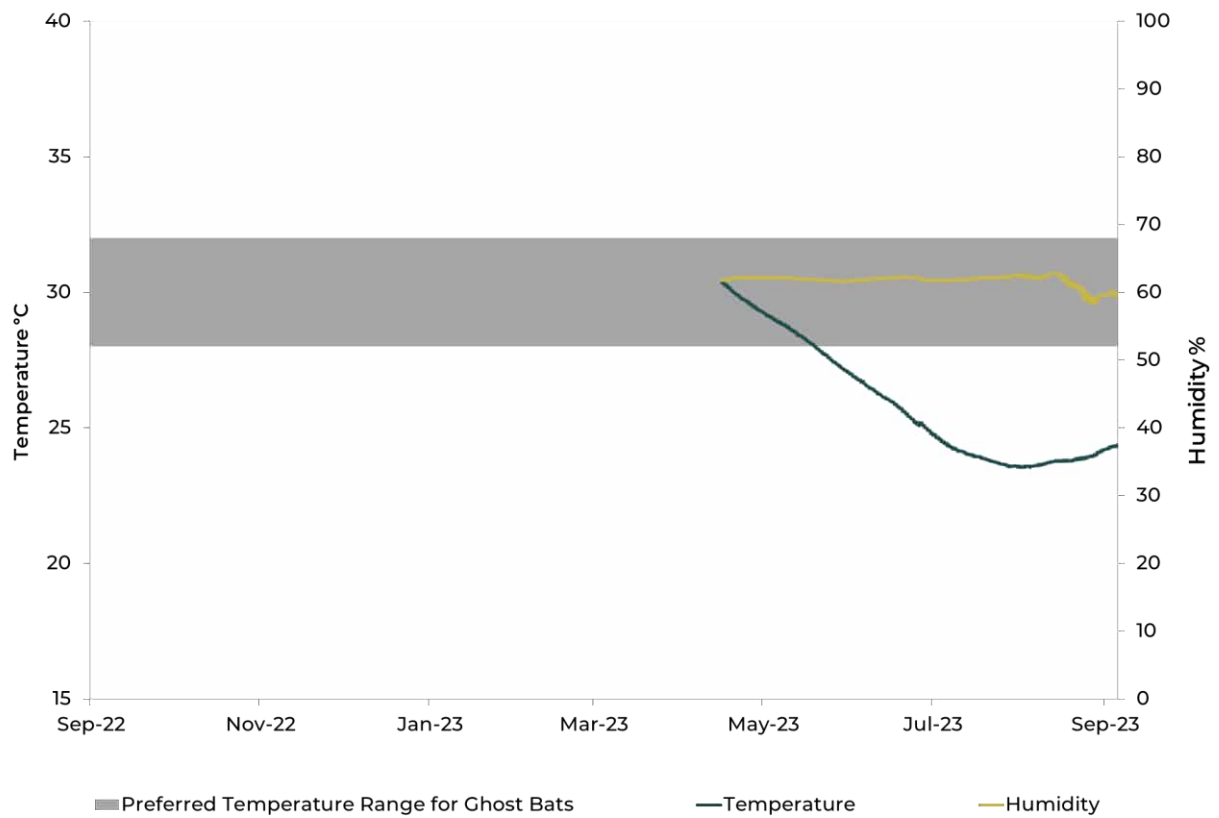




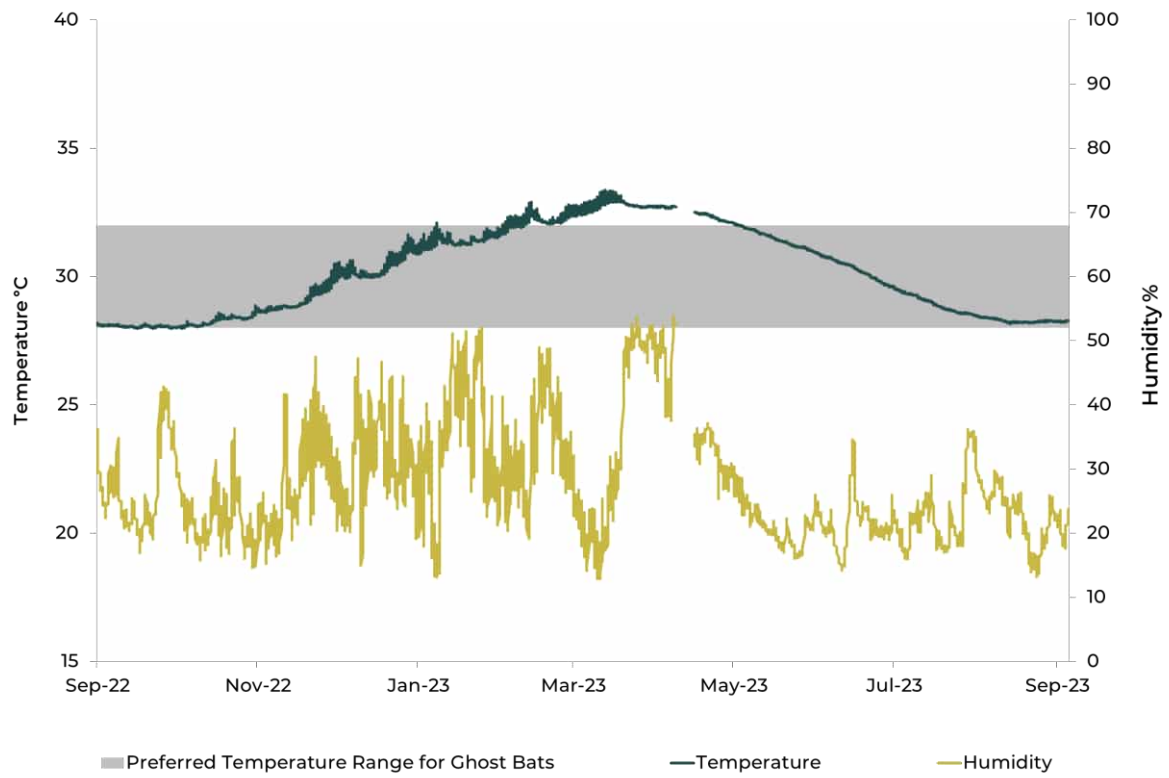
CJIM-07



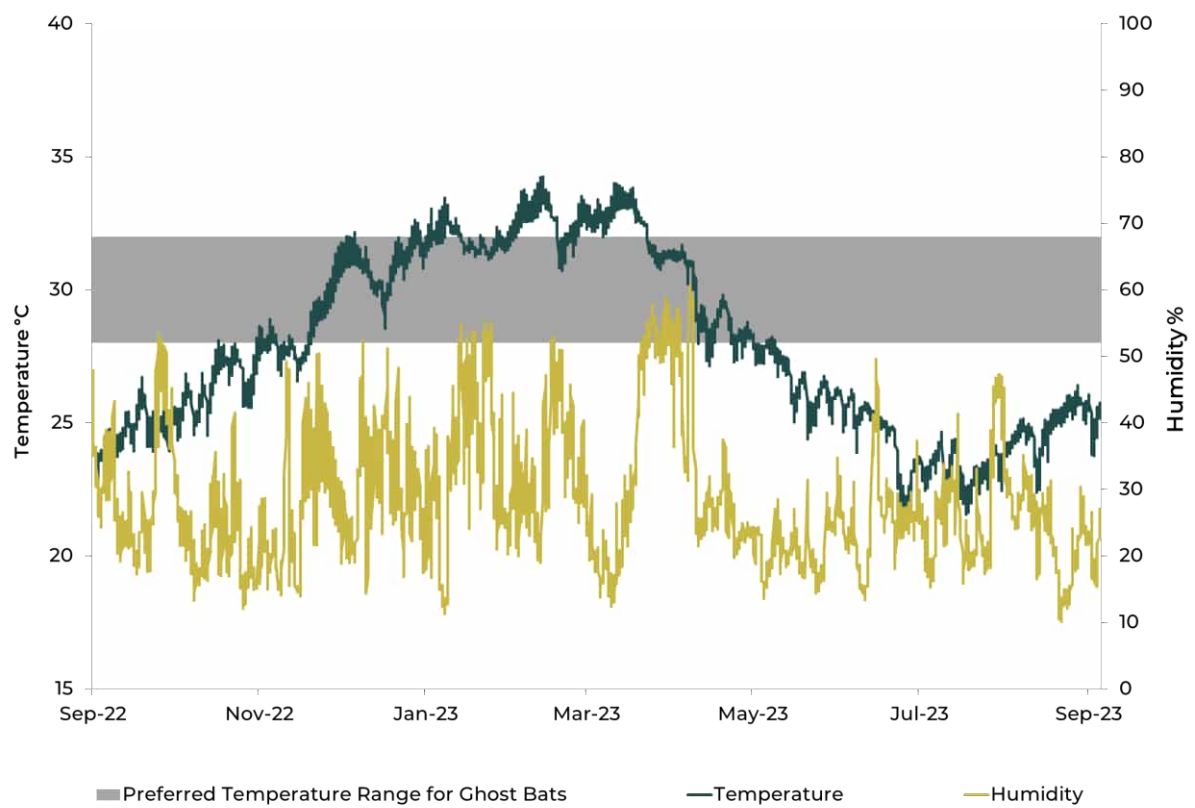
CJIM-08



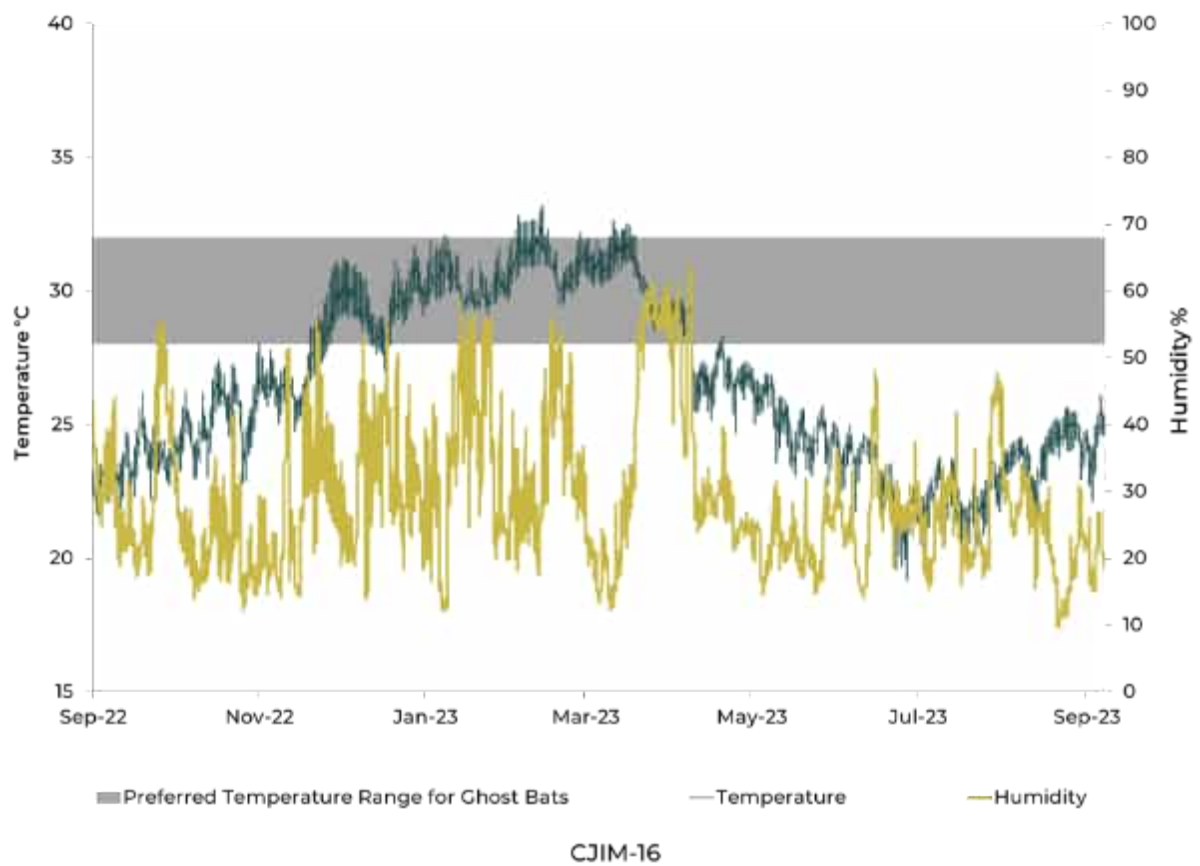
CJIM-09

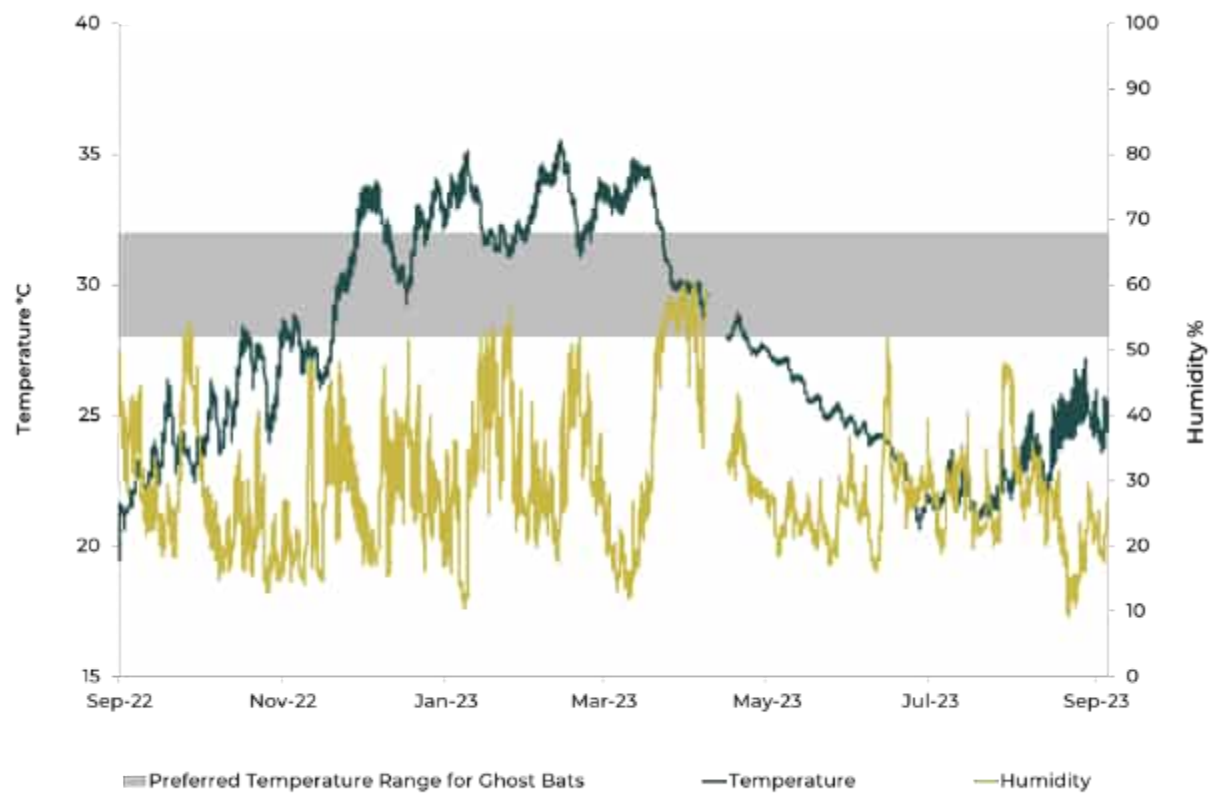


CJIM-14

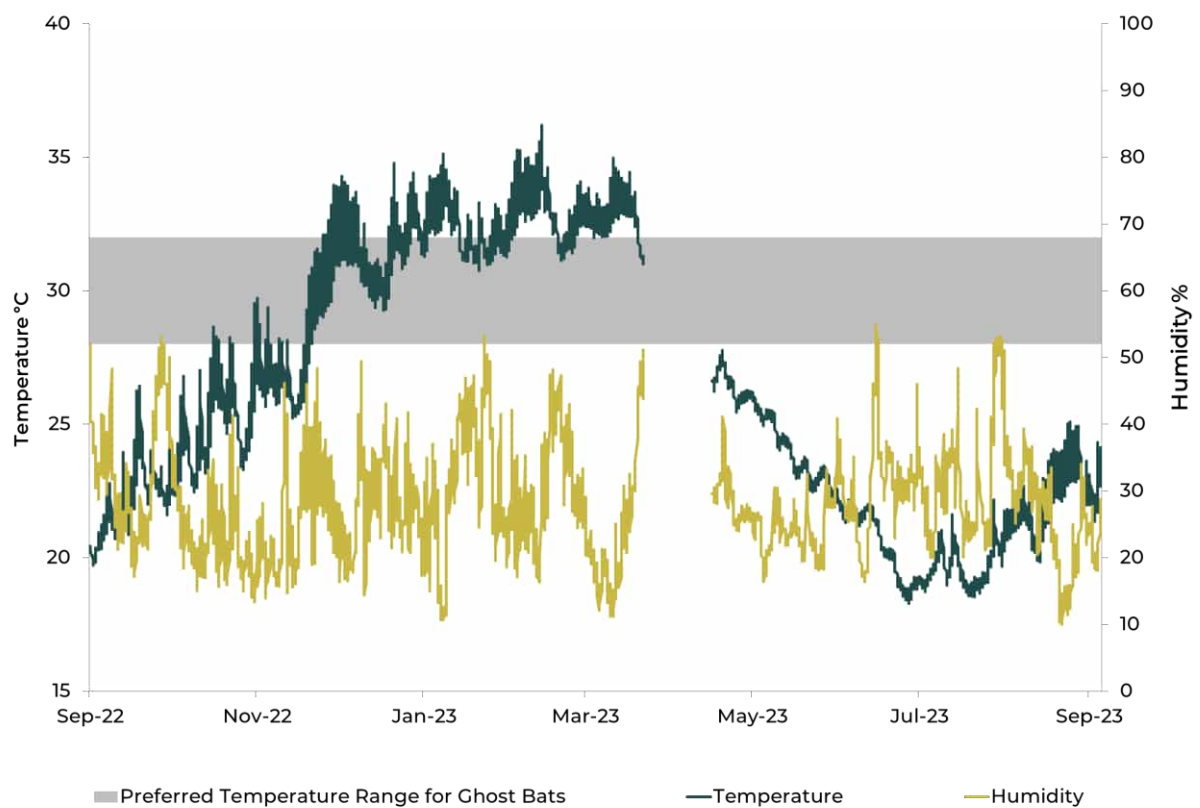


CJIM-15

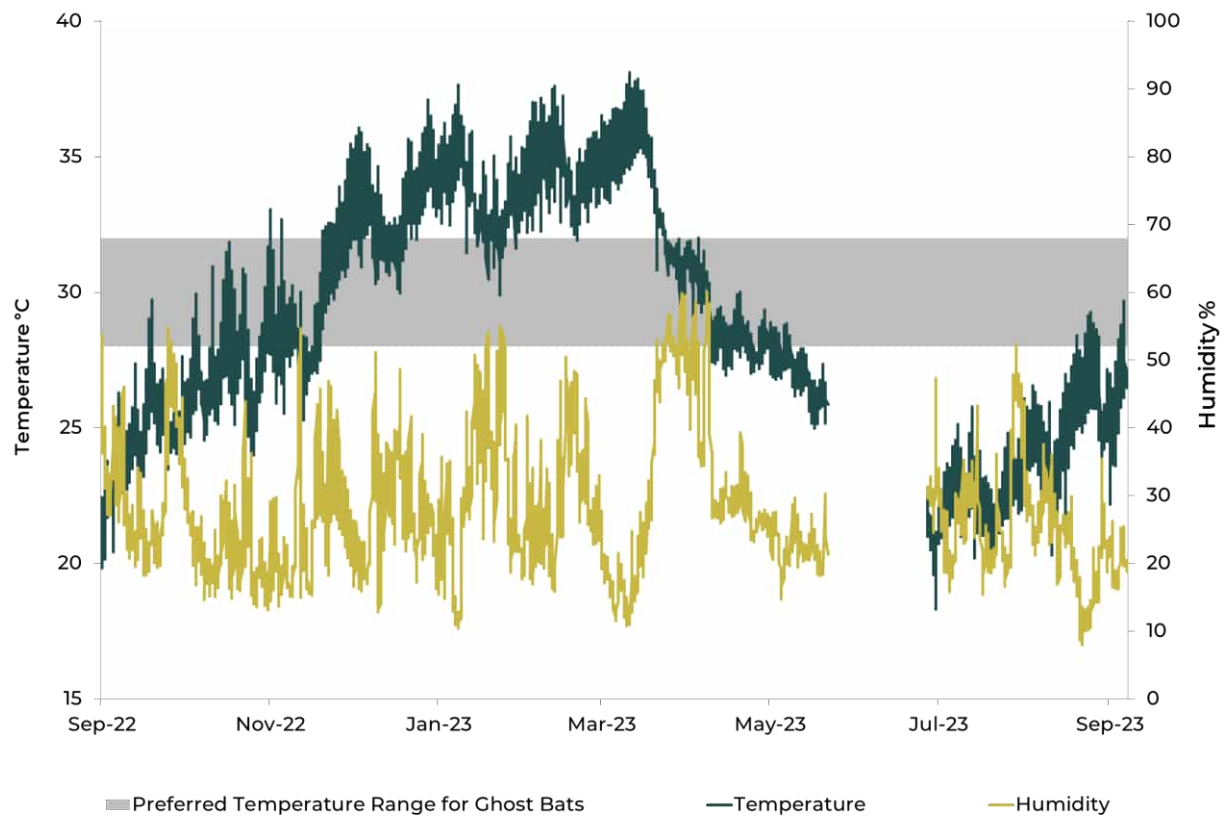




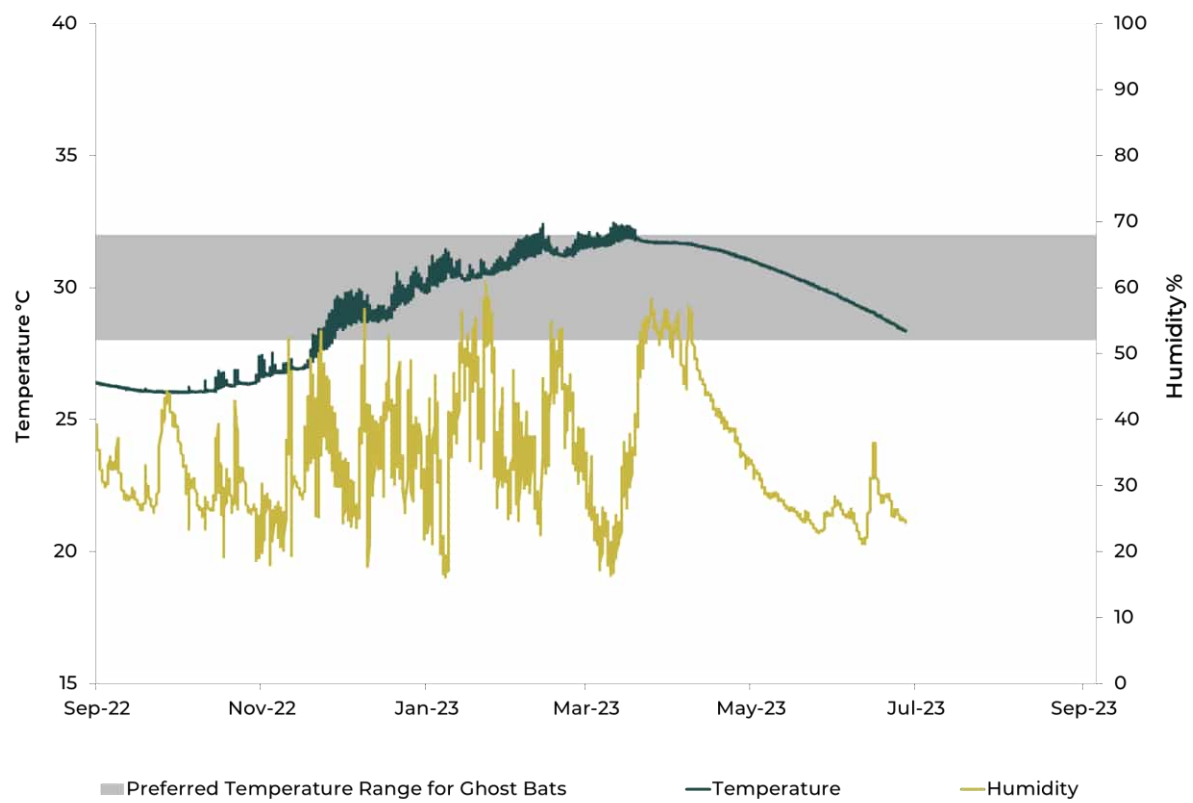
CJIM-17



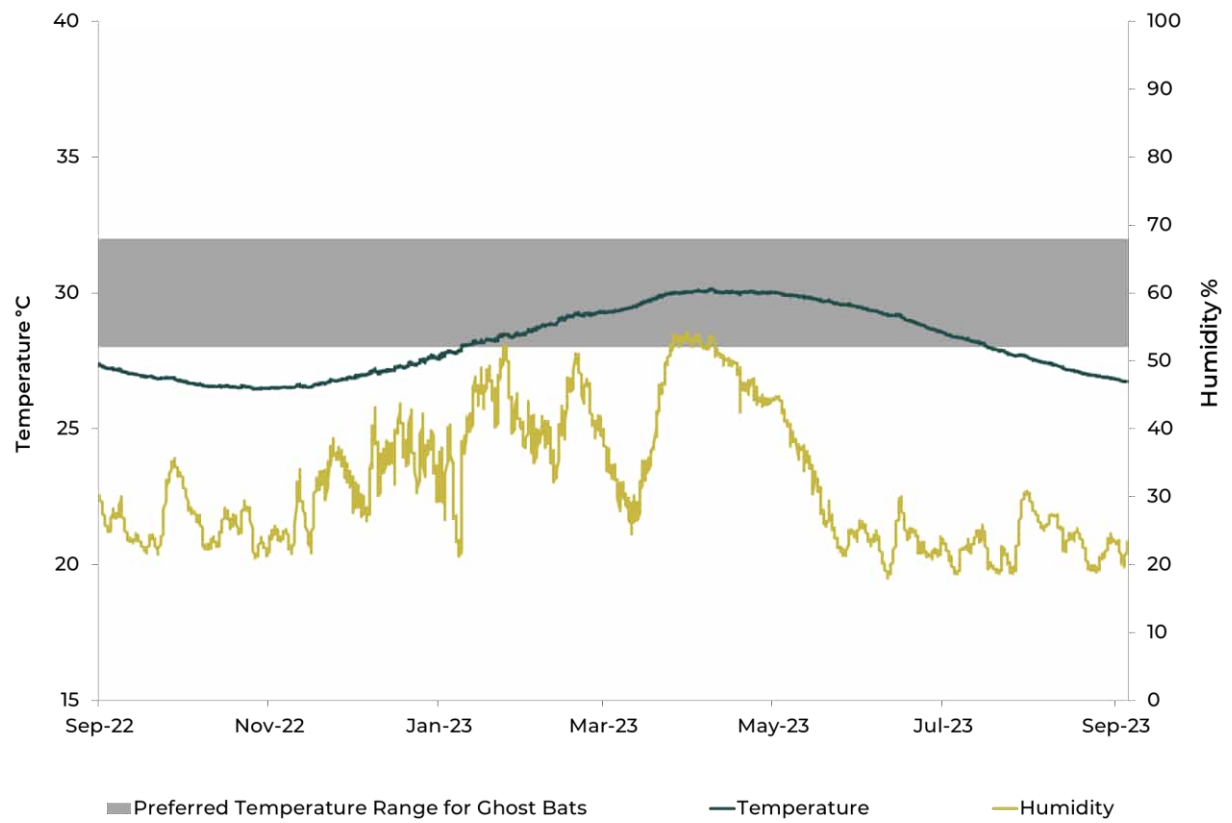
CJIM-18



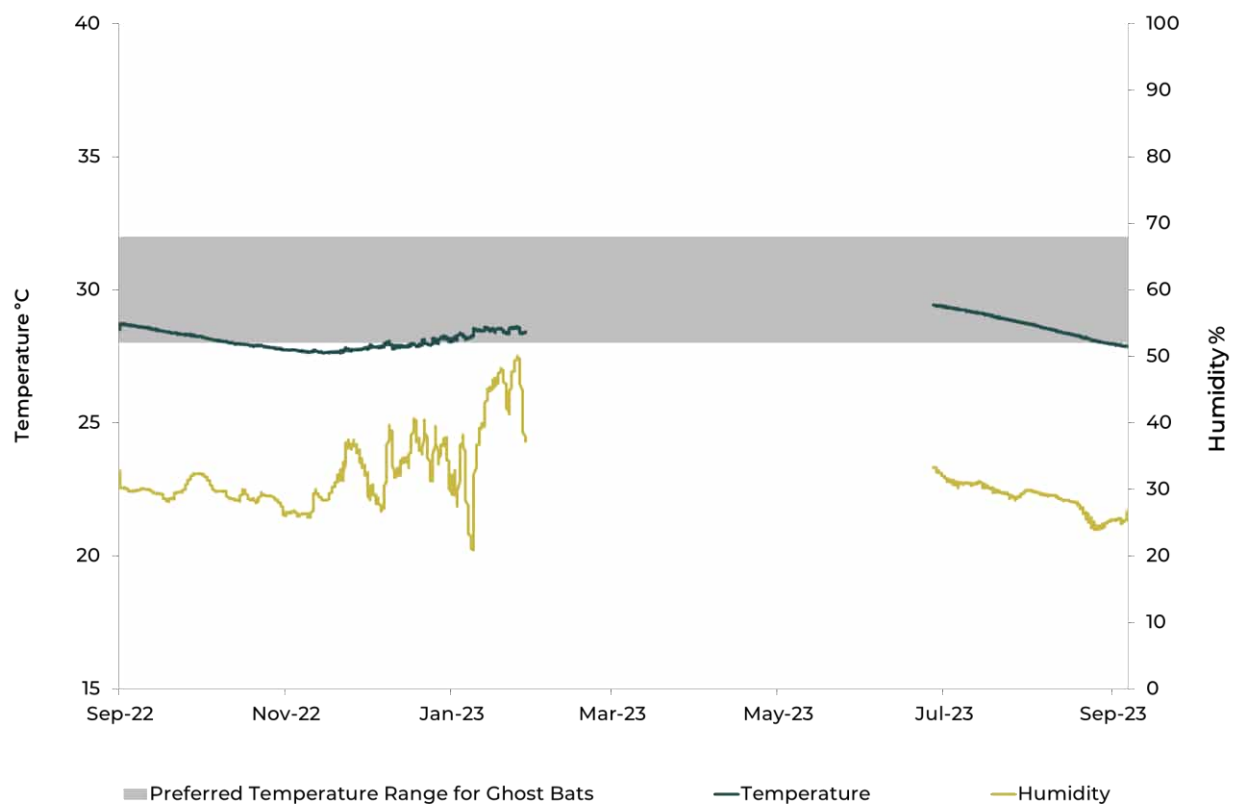
CJIM-20



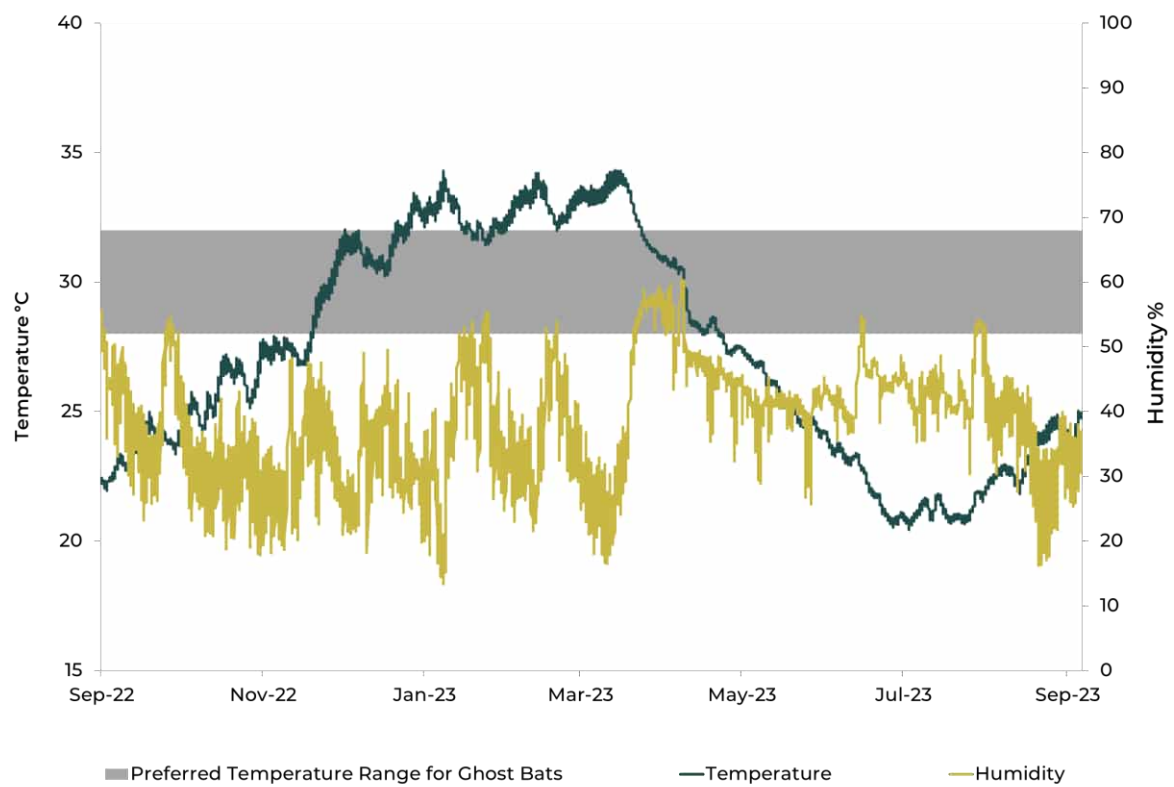
CNIN-01



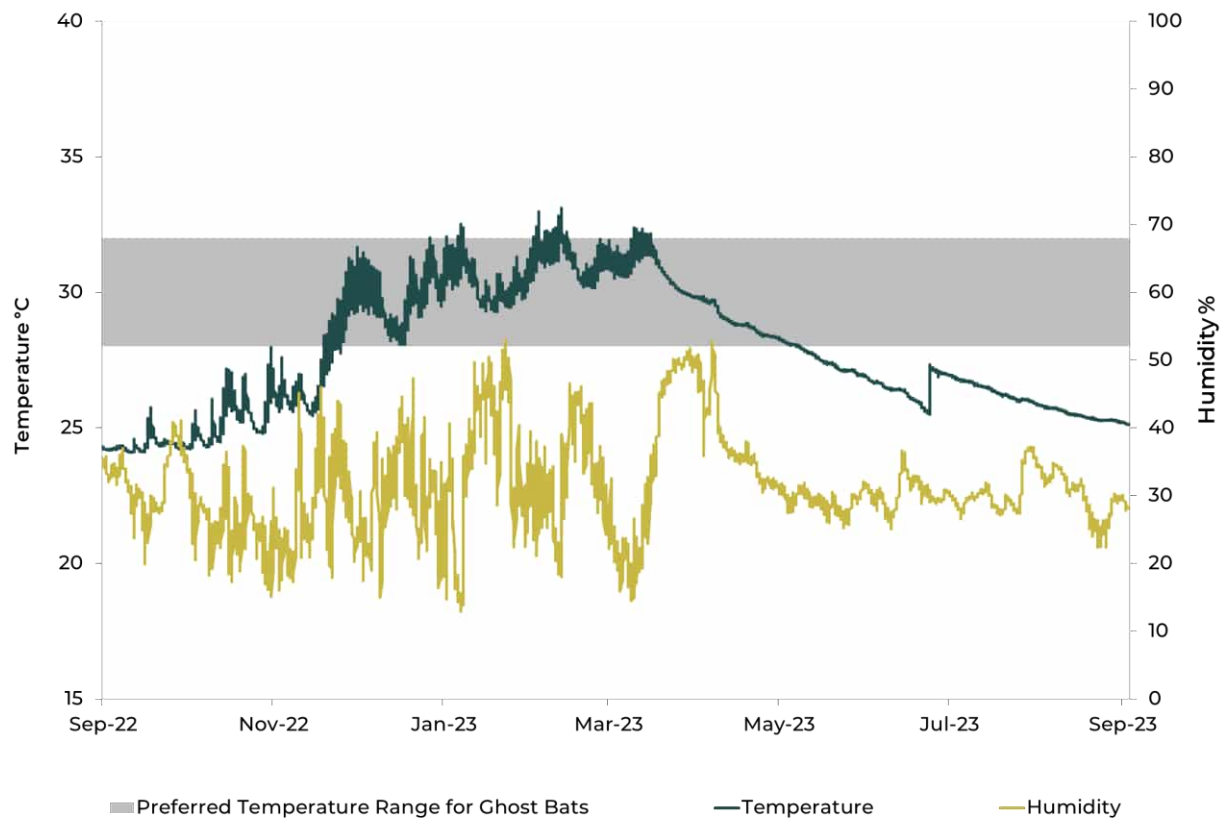
CNIN-02



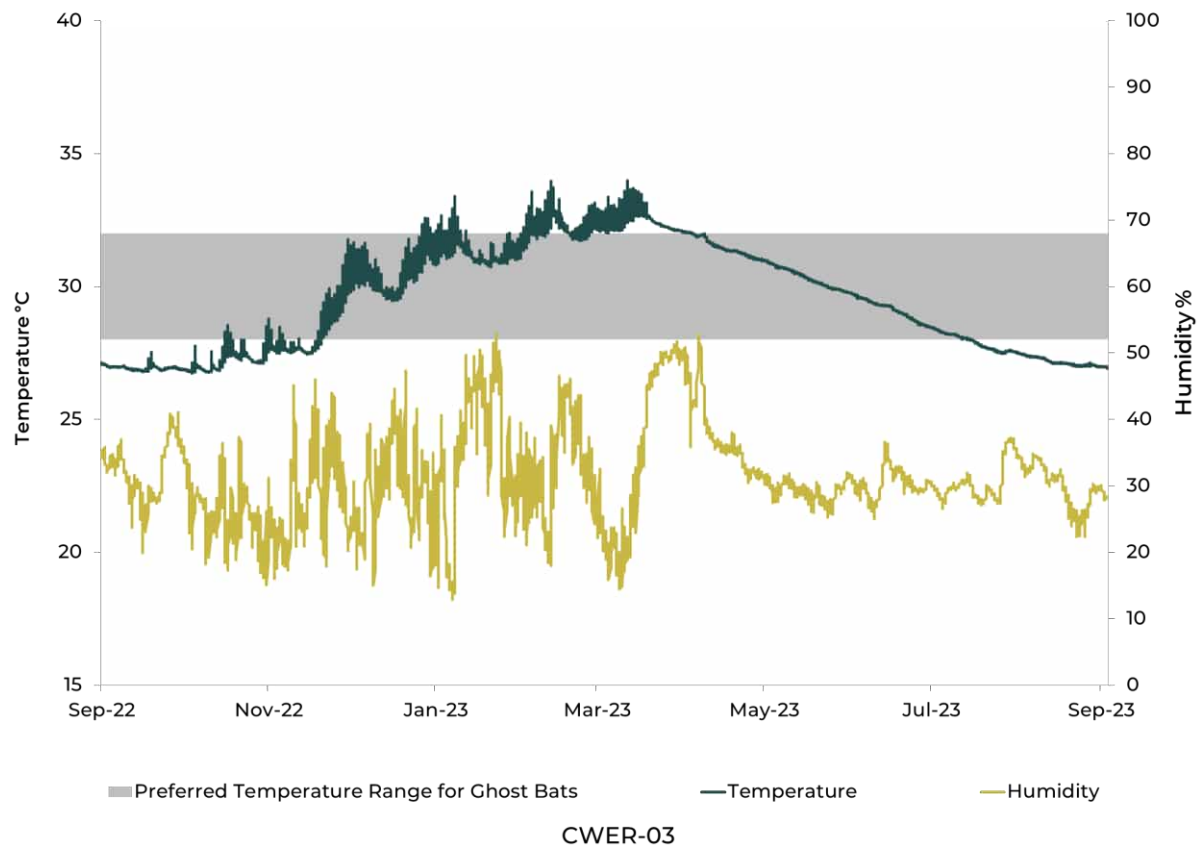
CNIN-03

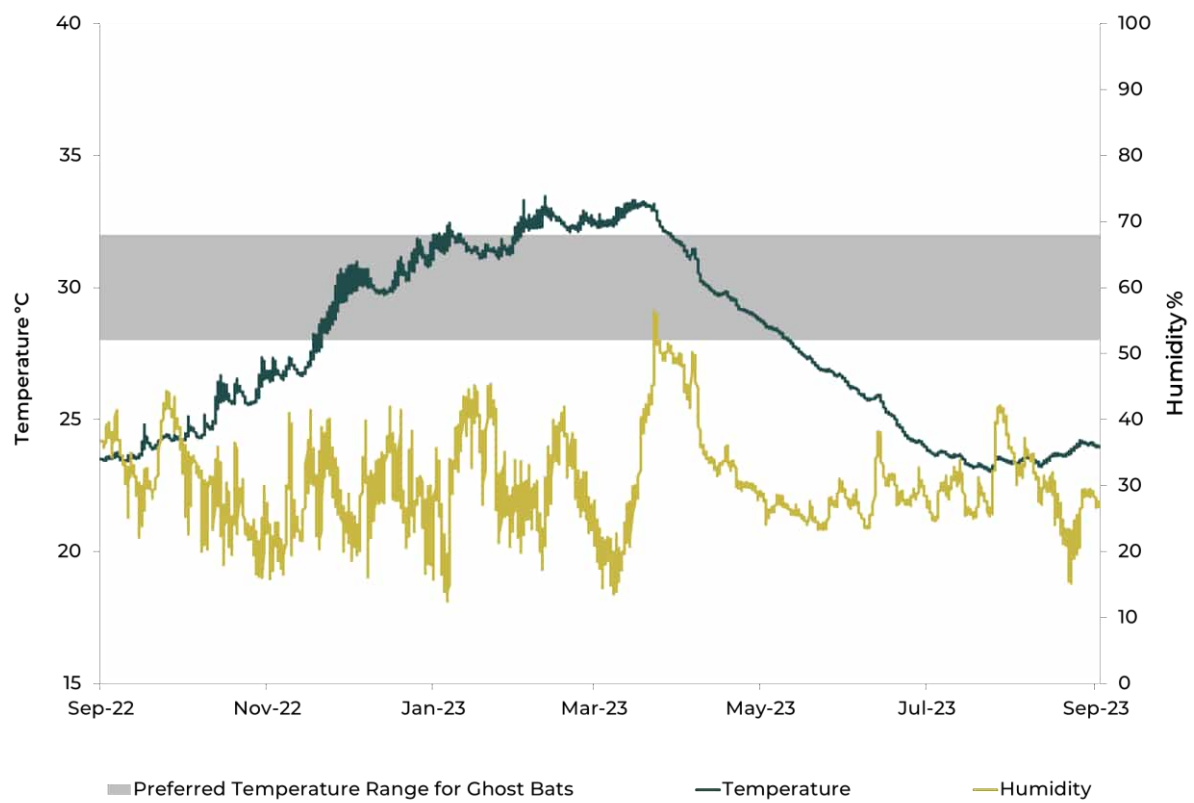


CNIN-13

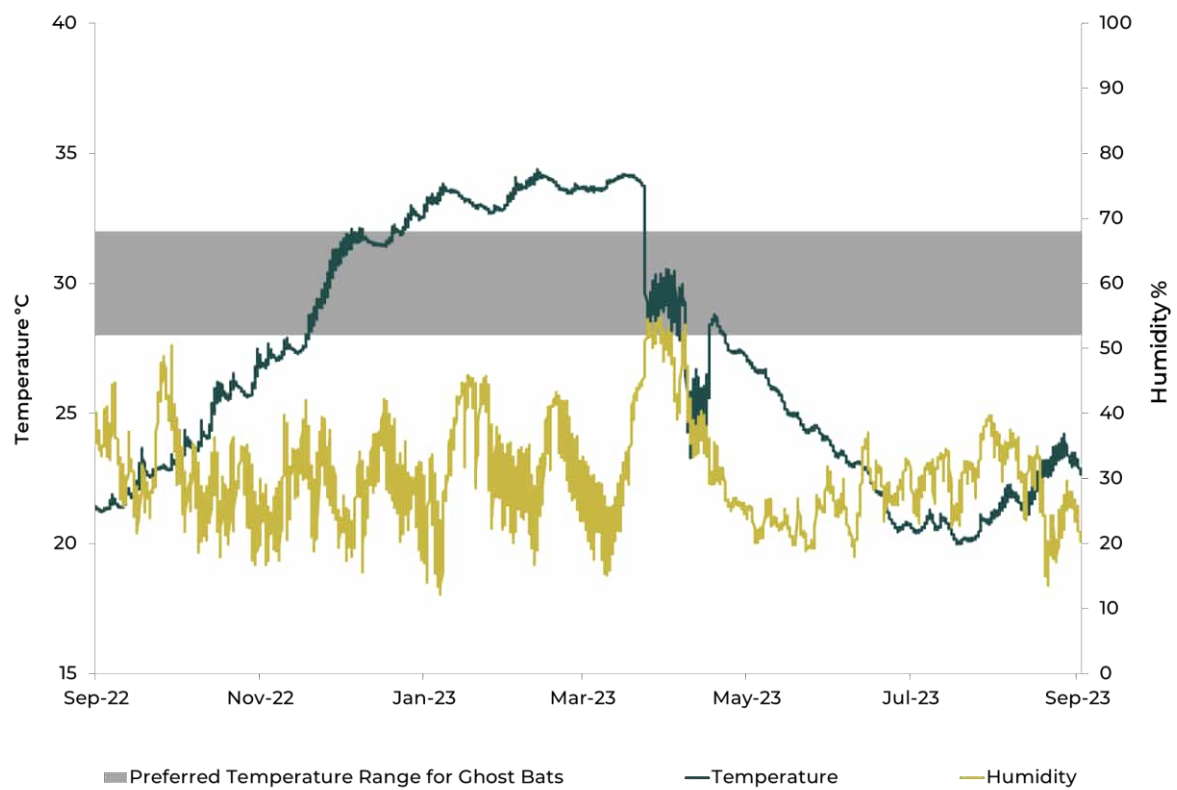


CWER-01

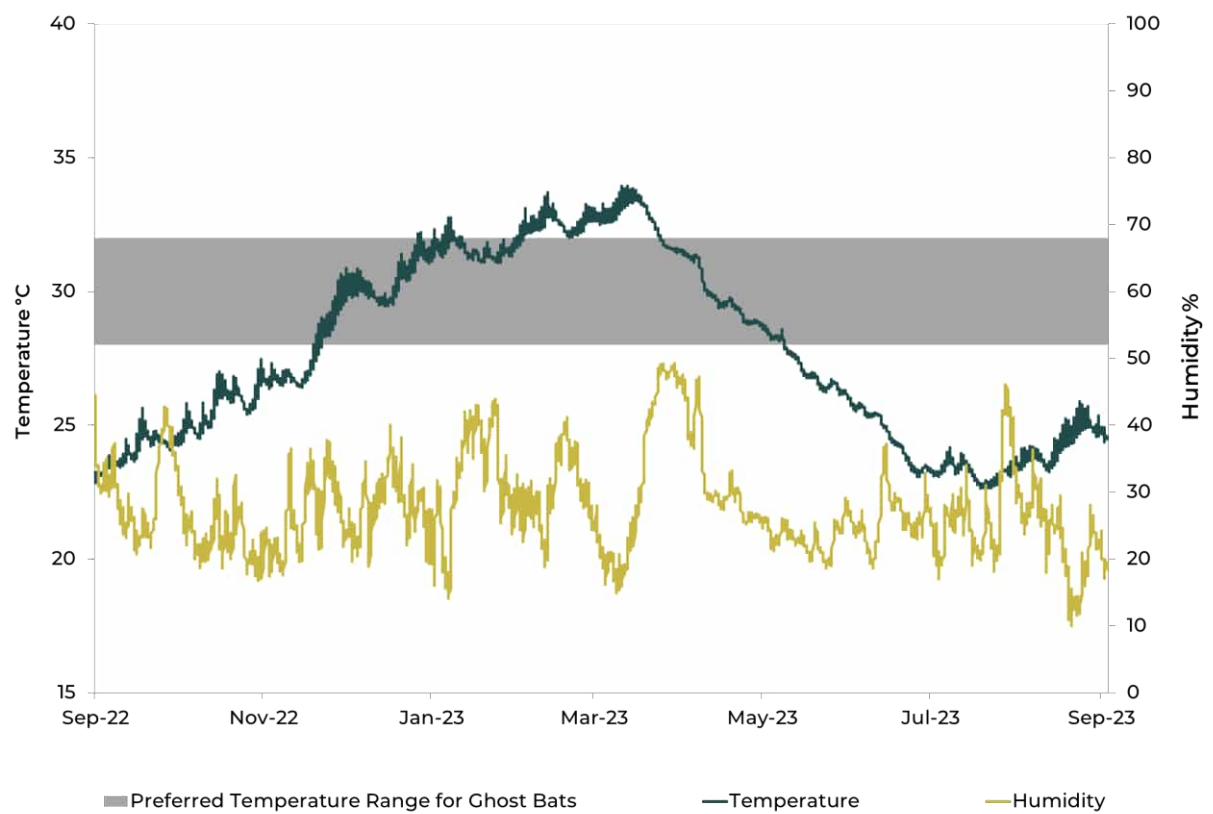




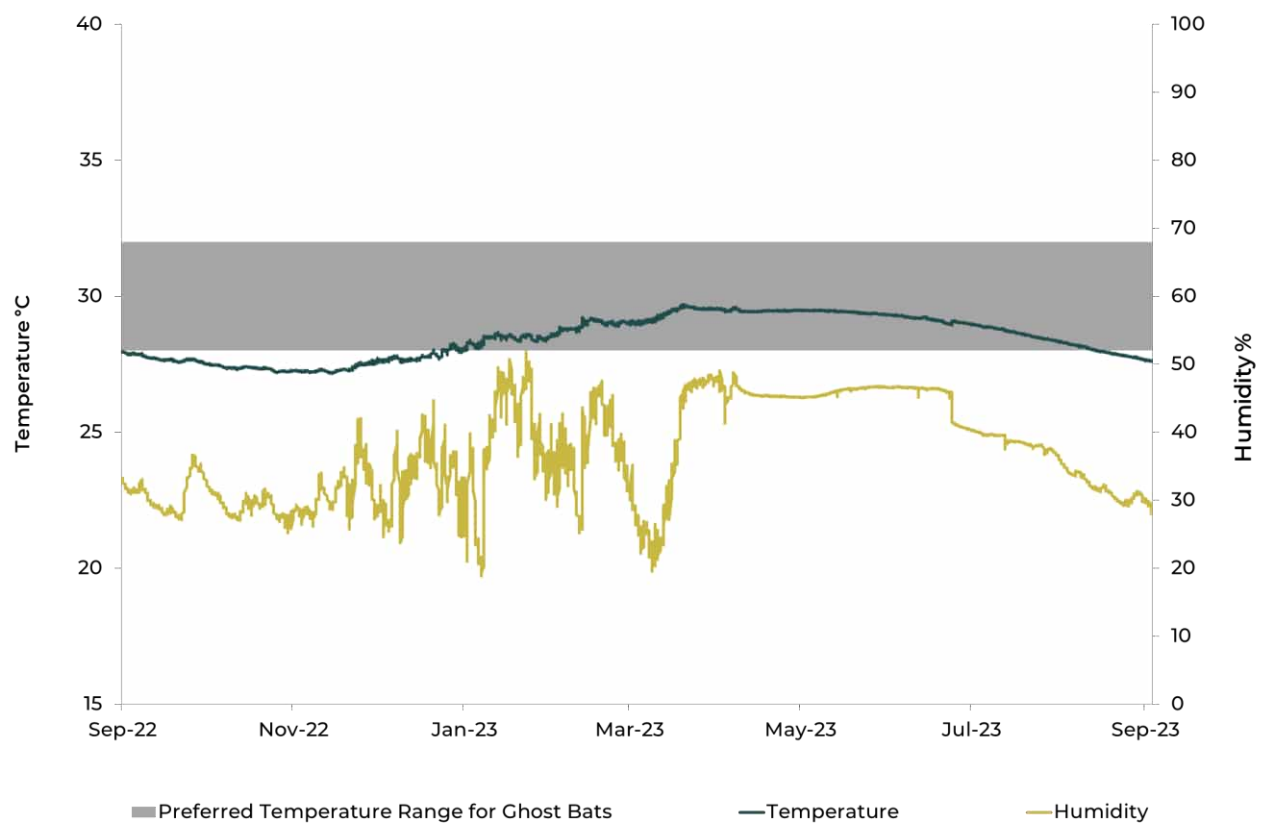
CWER-04



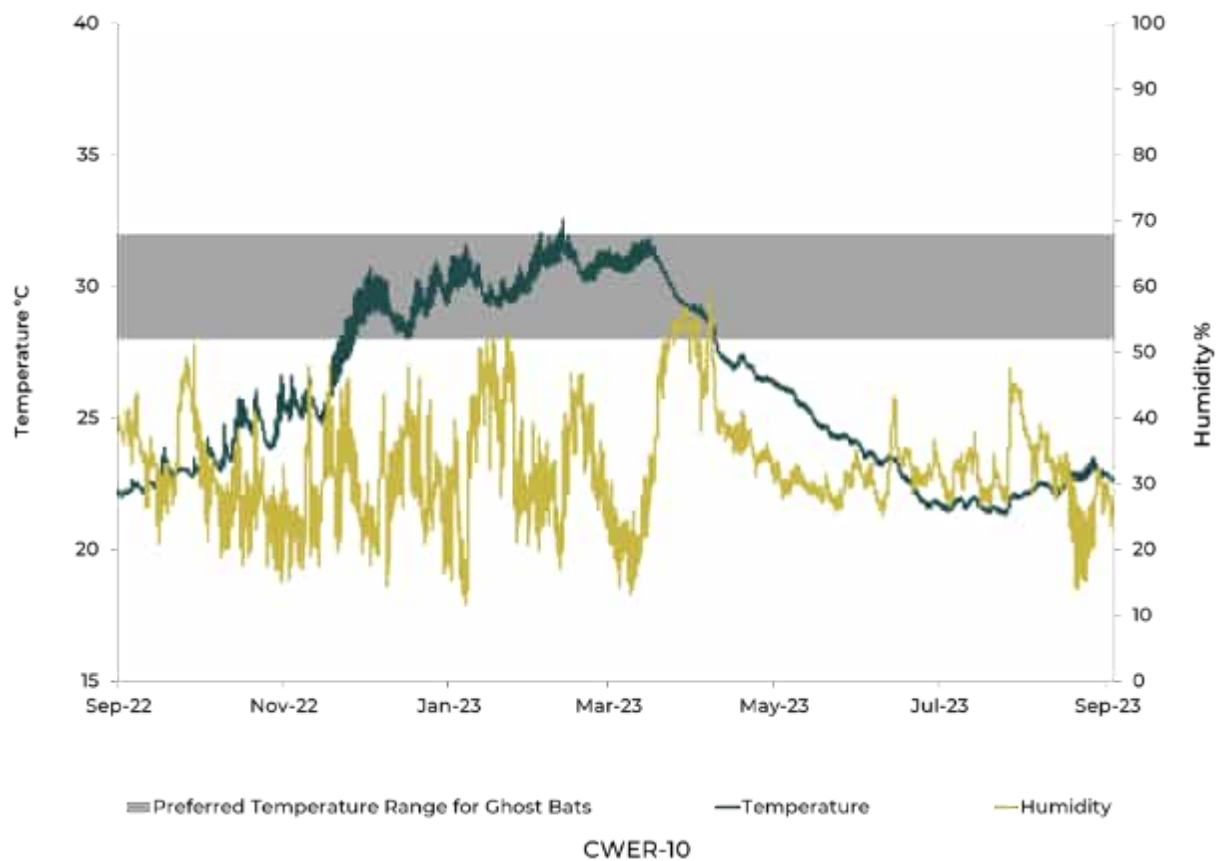
CWER-05

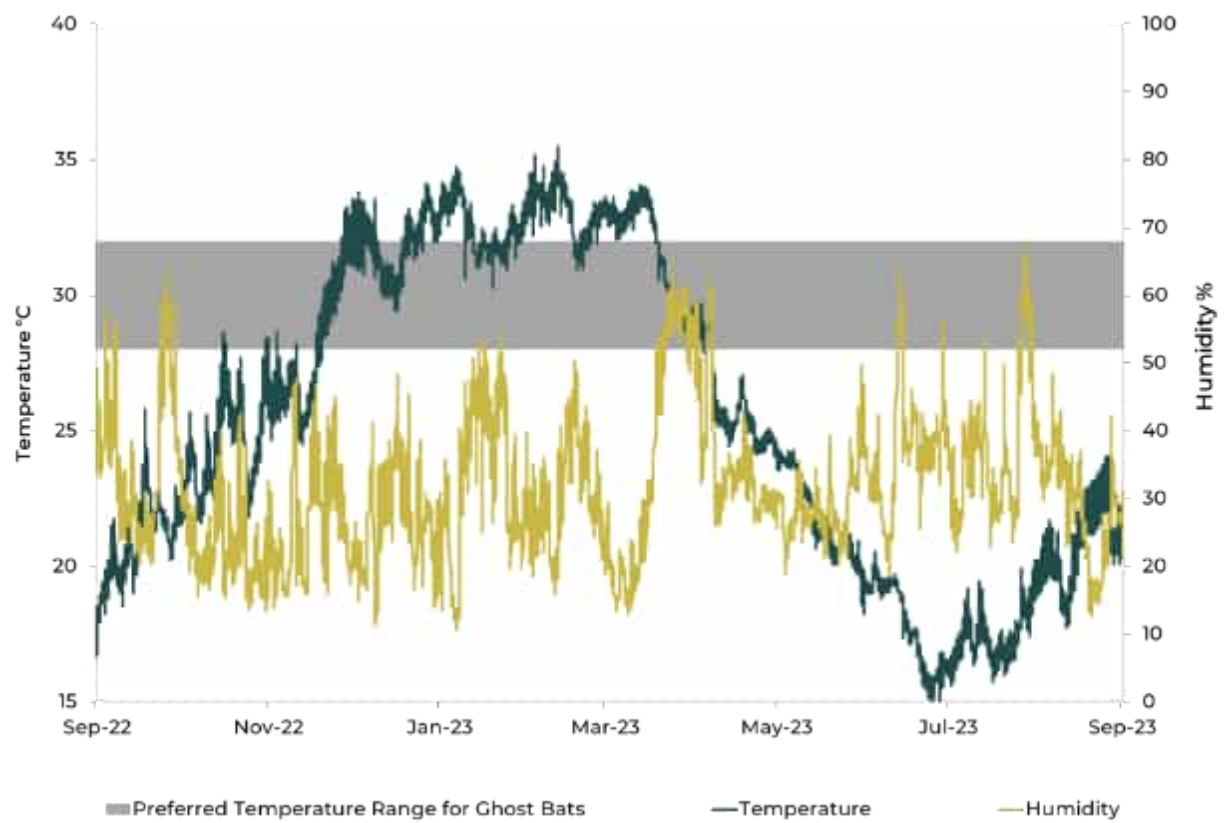


CWER-07

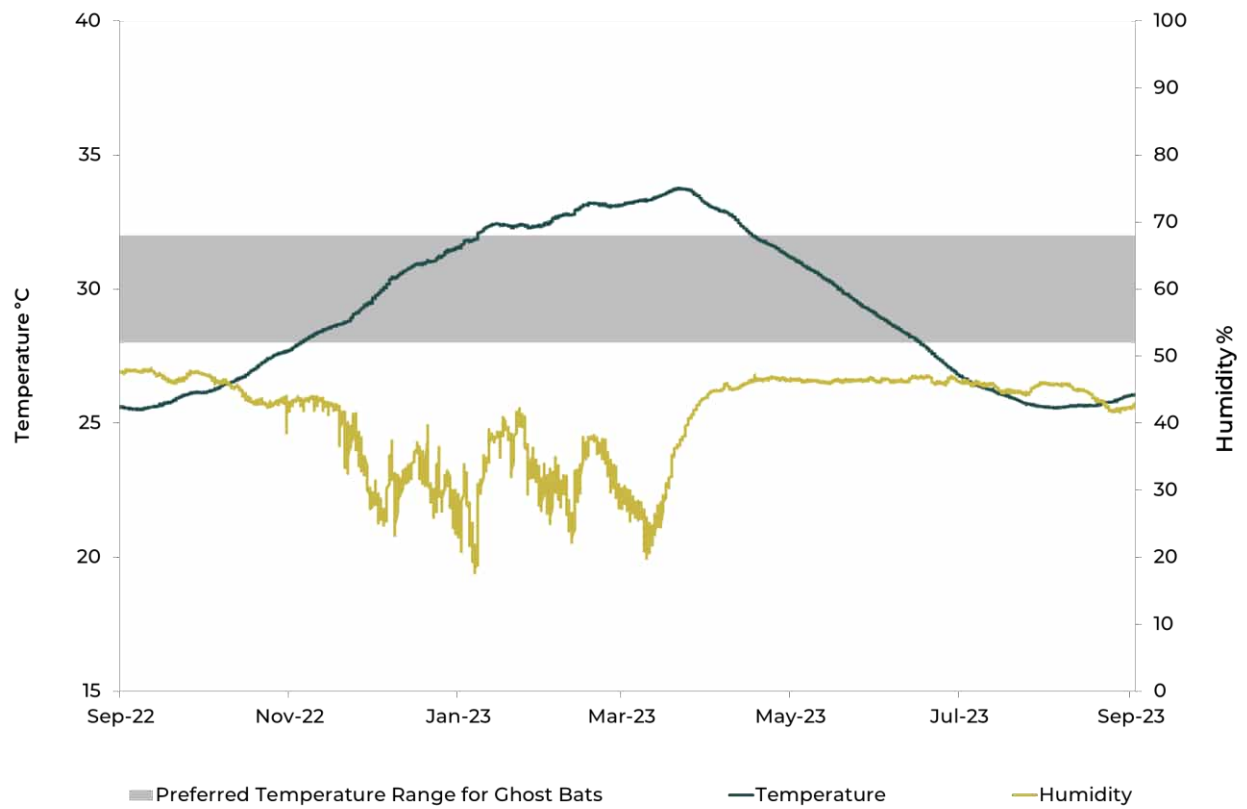


CWER-09

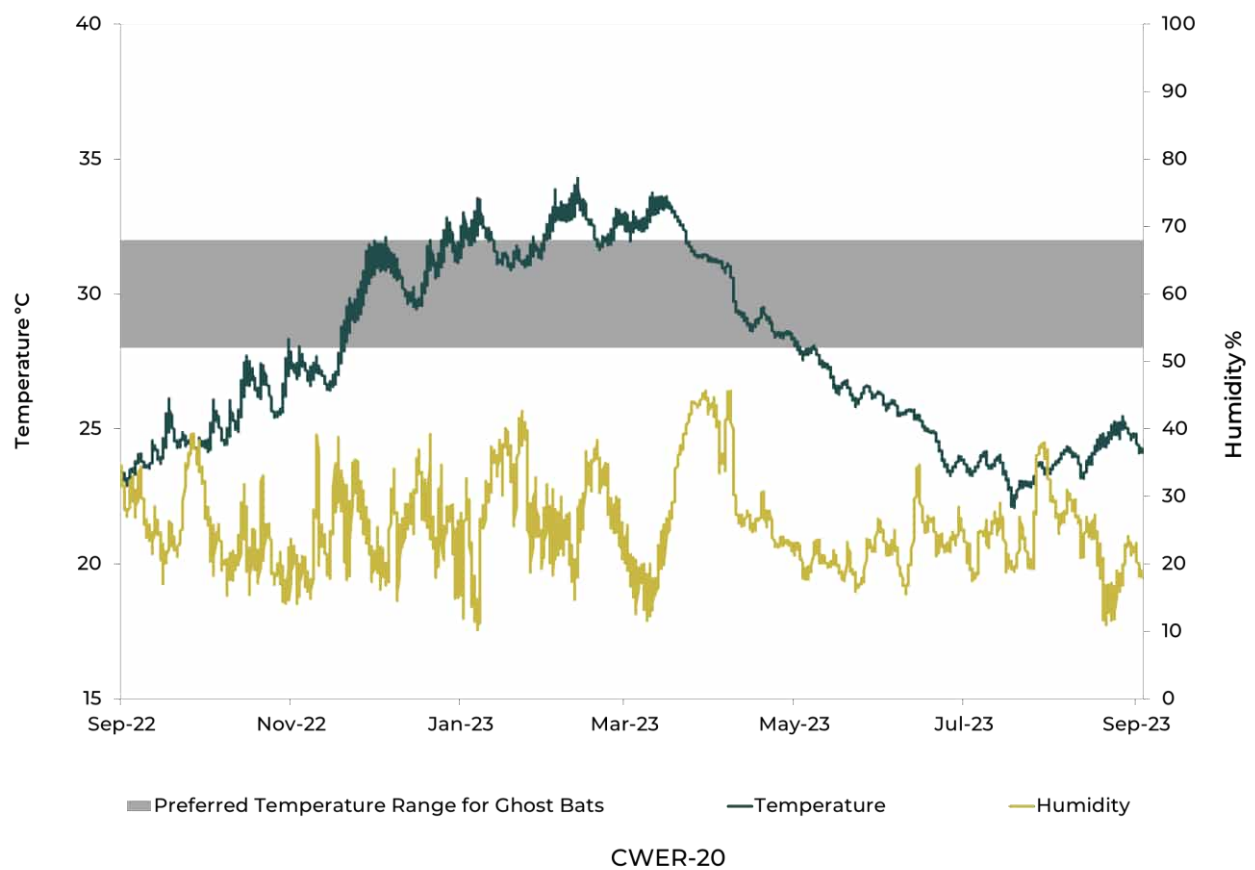




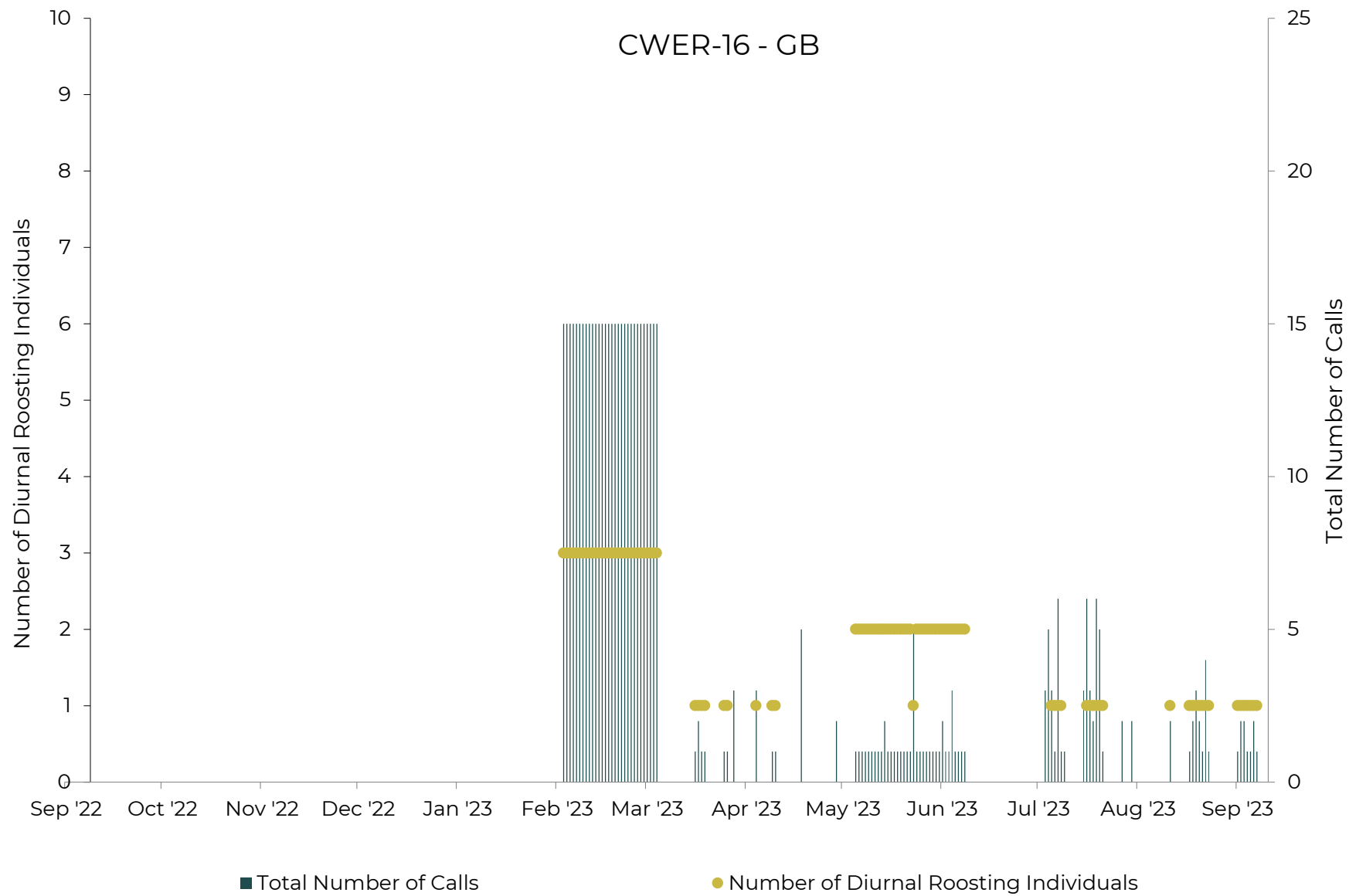
CWER-14

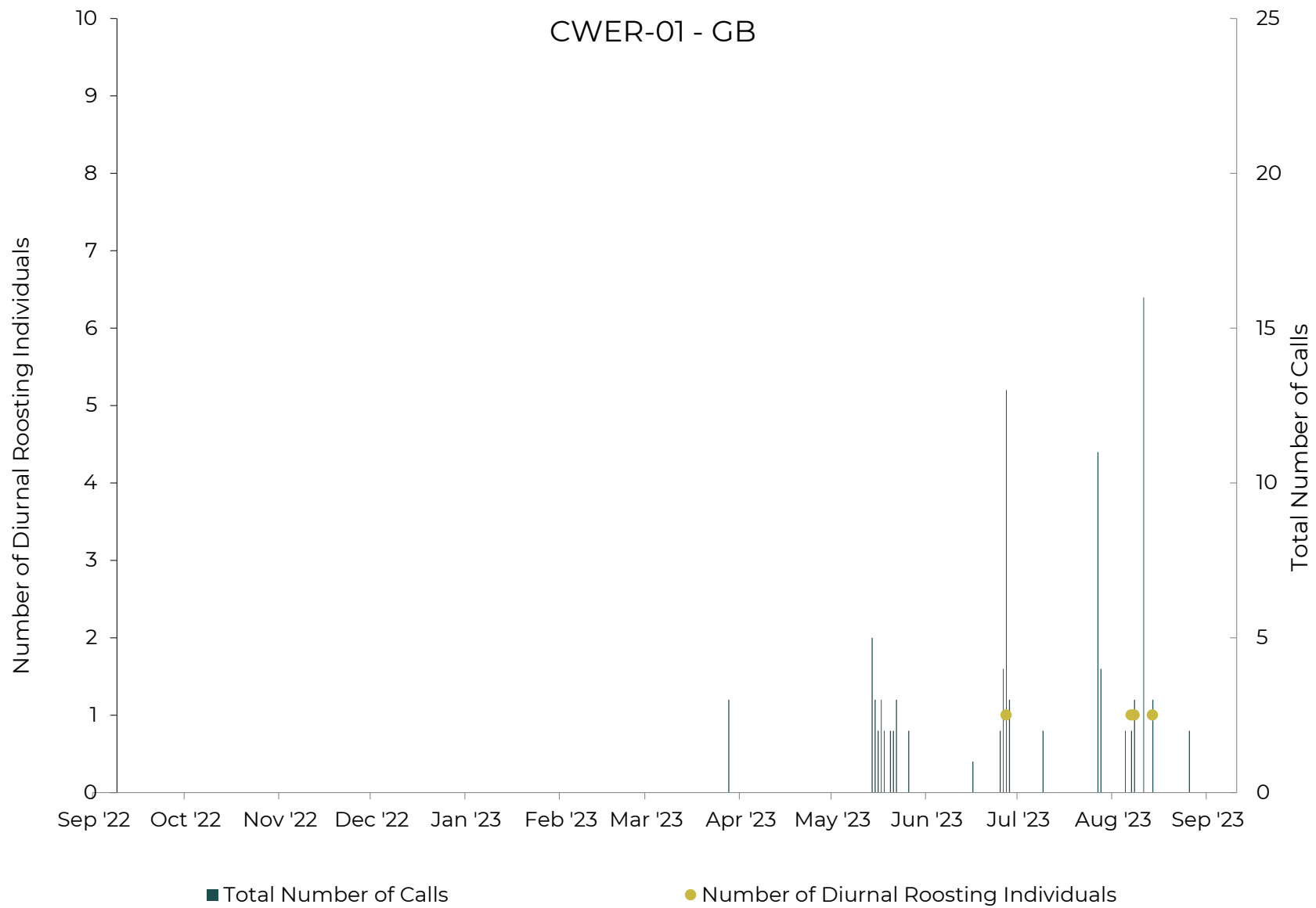


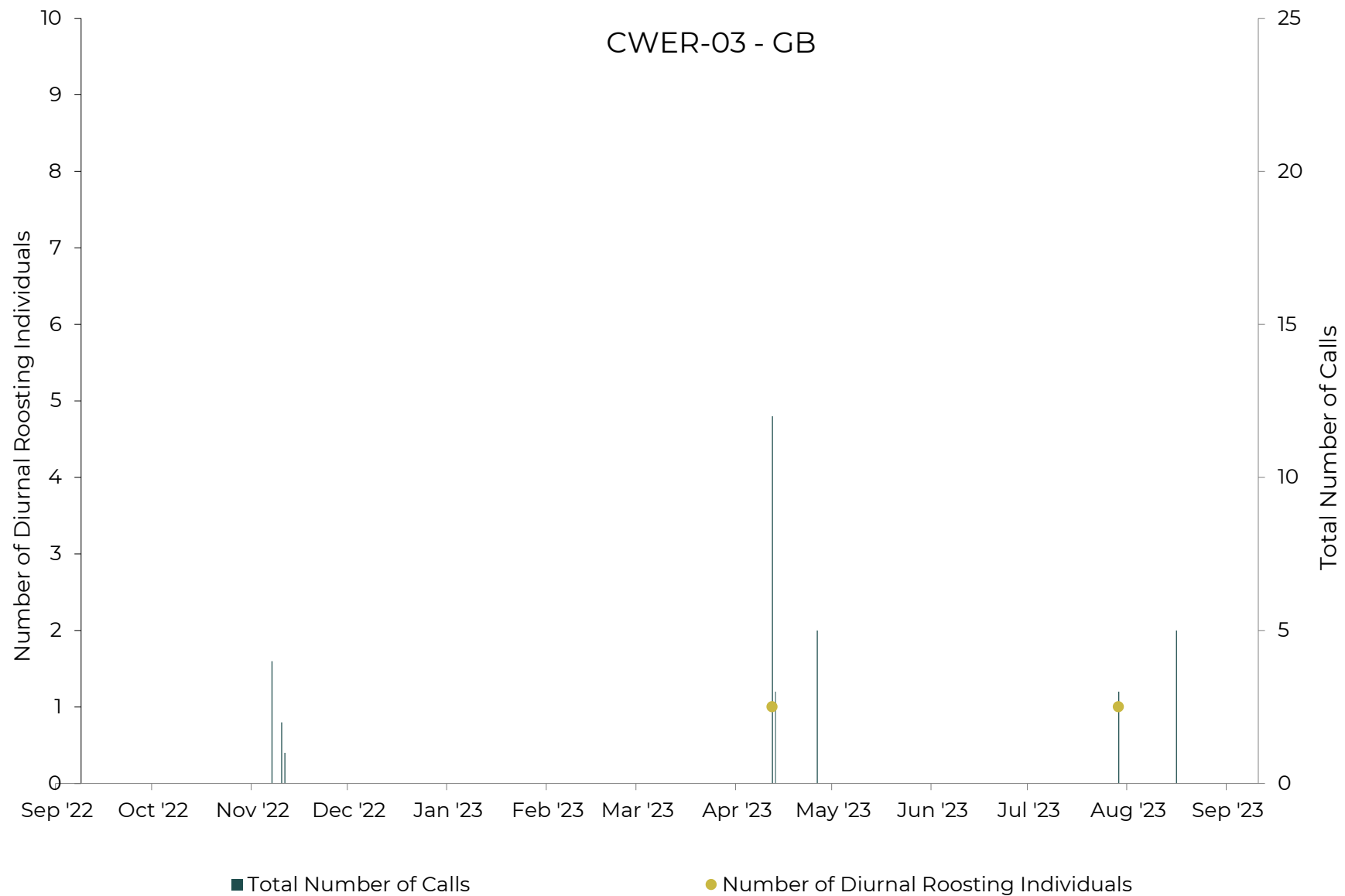
CWER-17

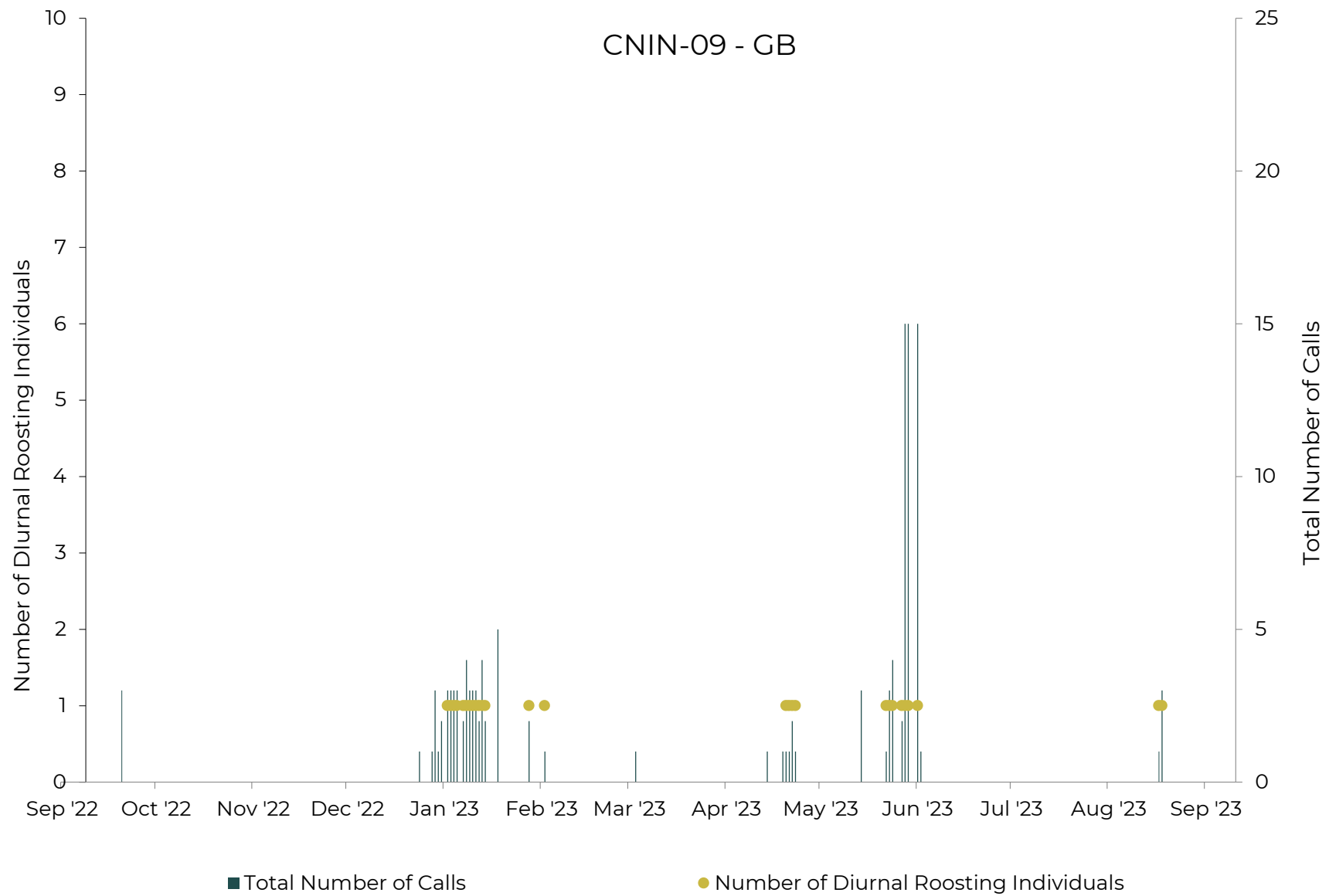


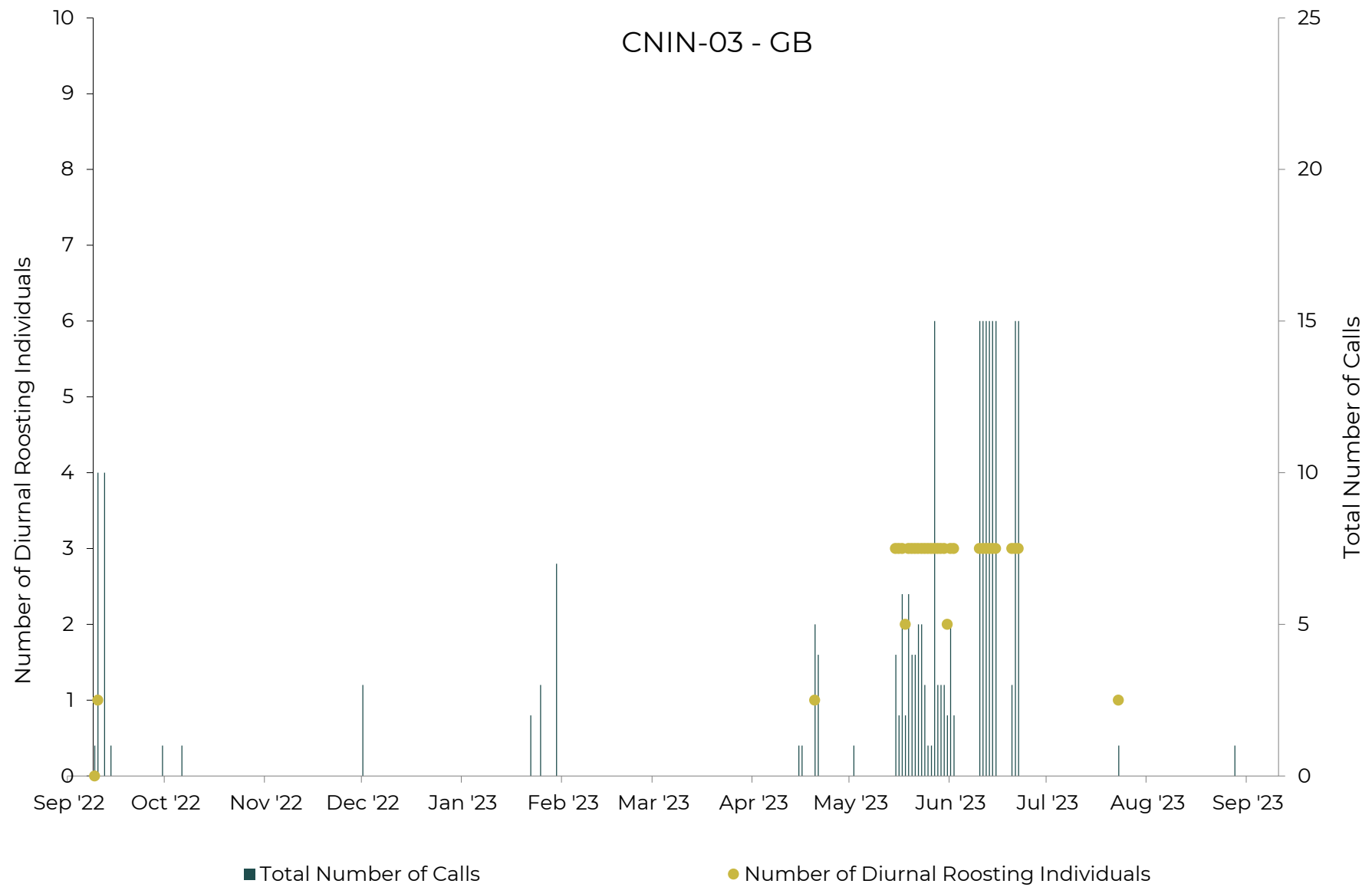
Appendix K: Ultrasonic Data

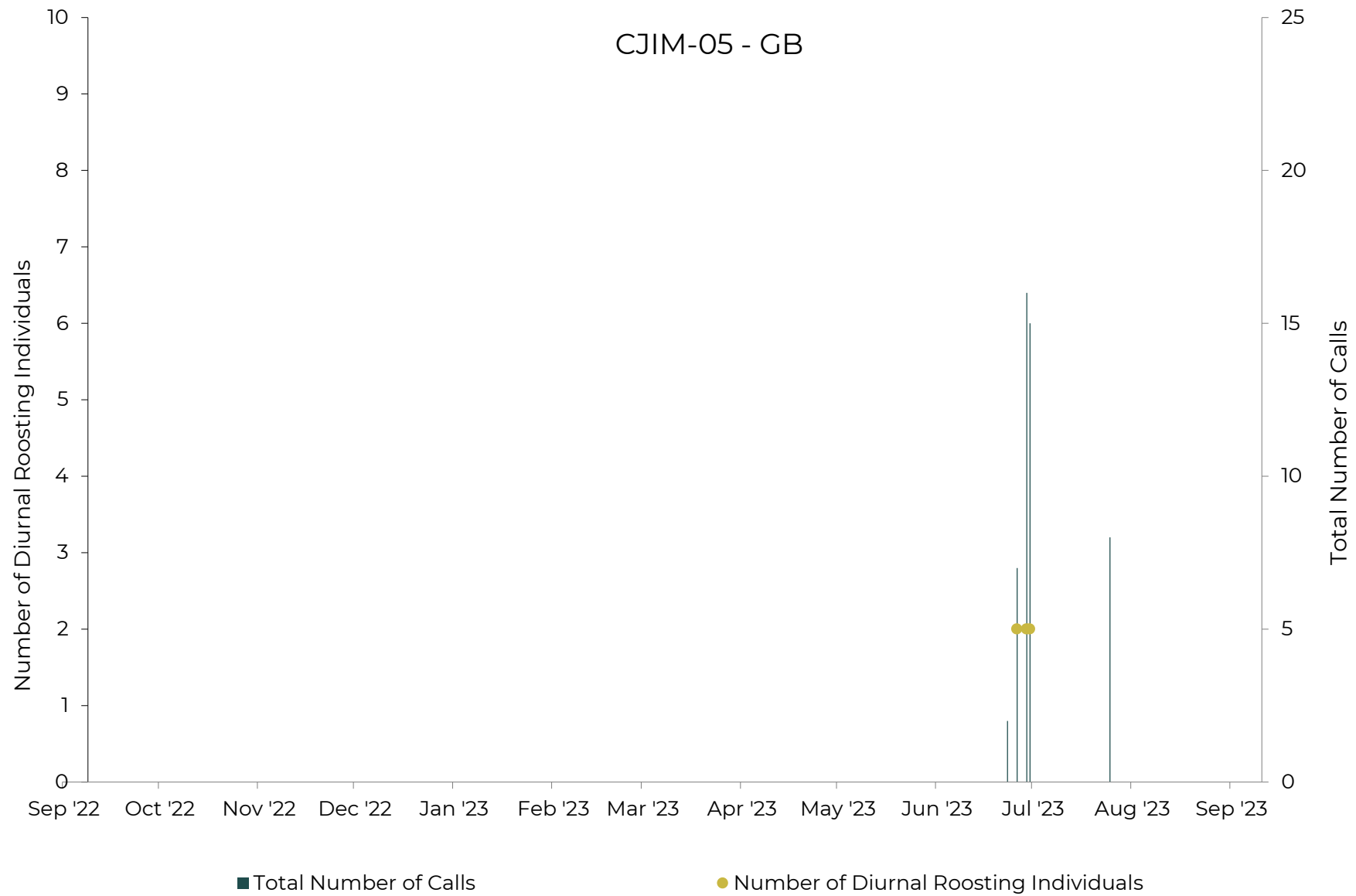




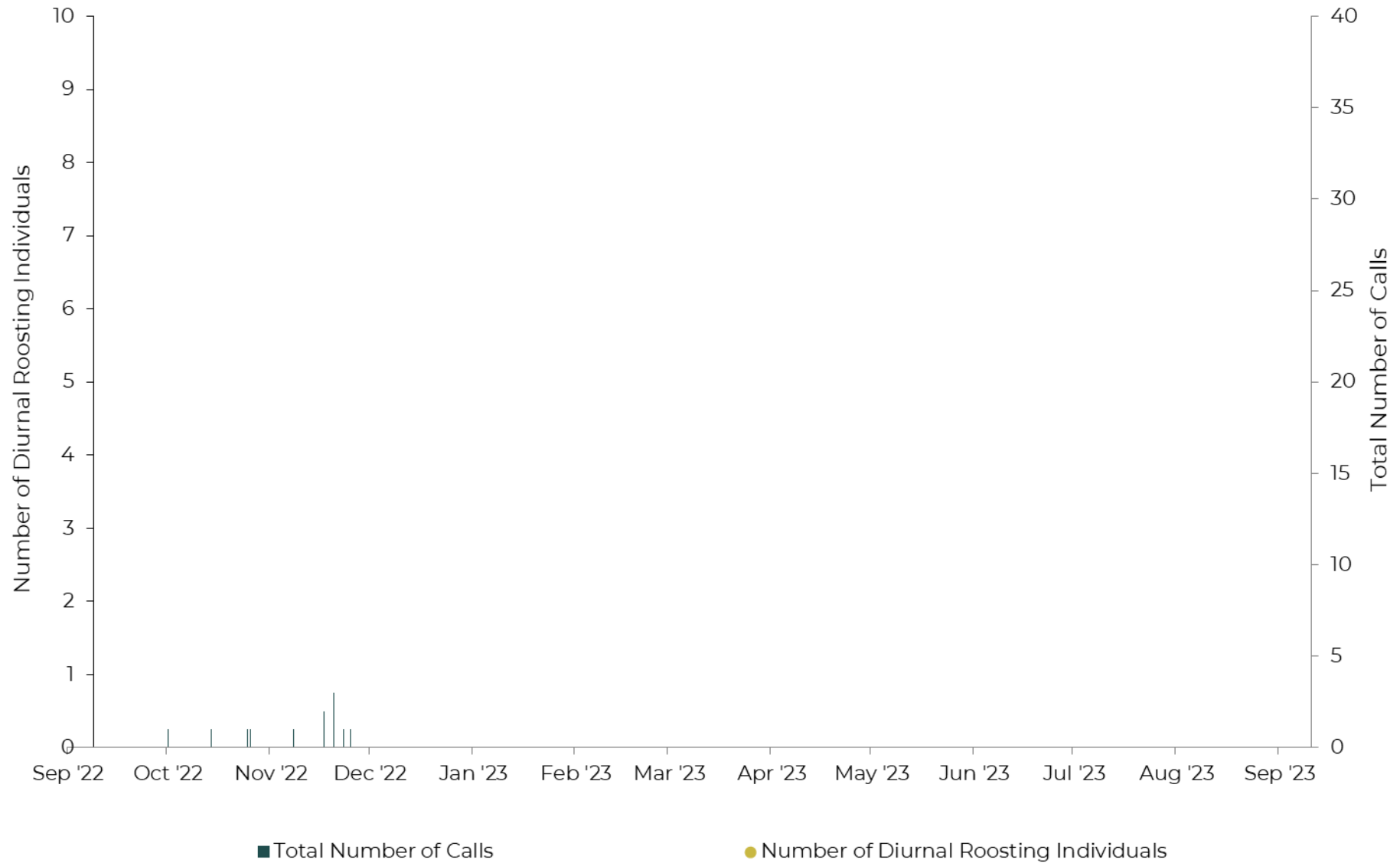




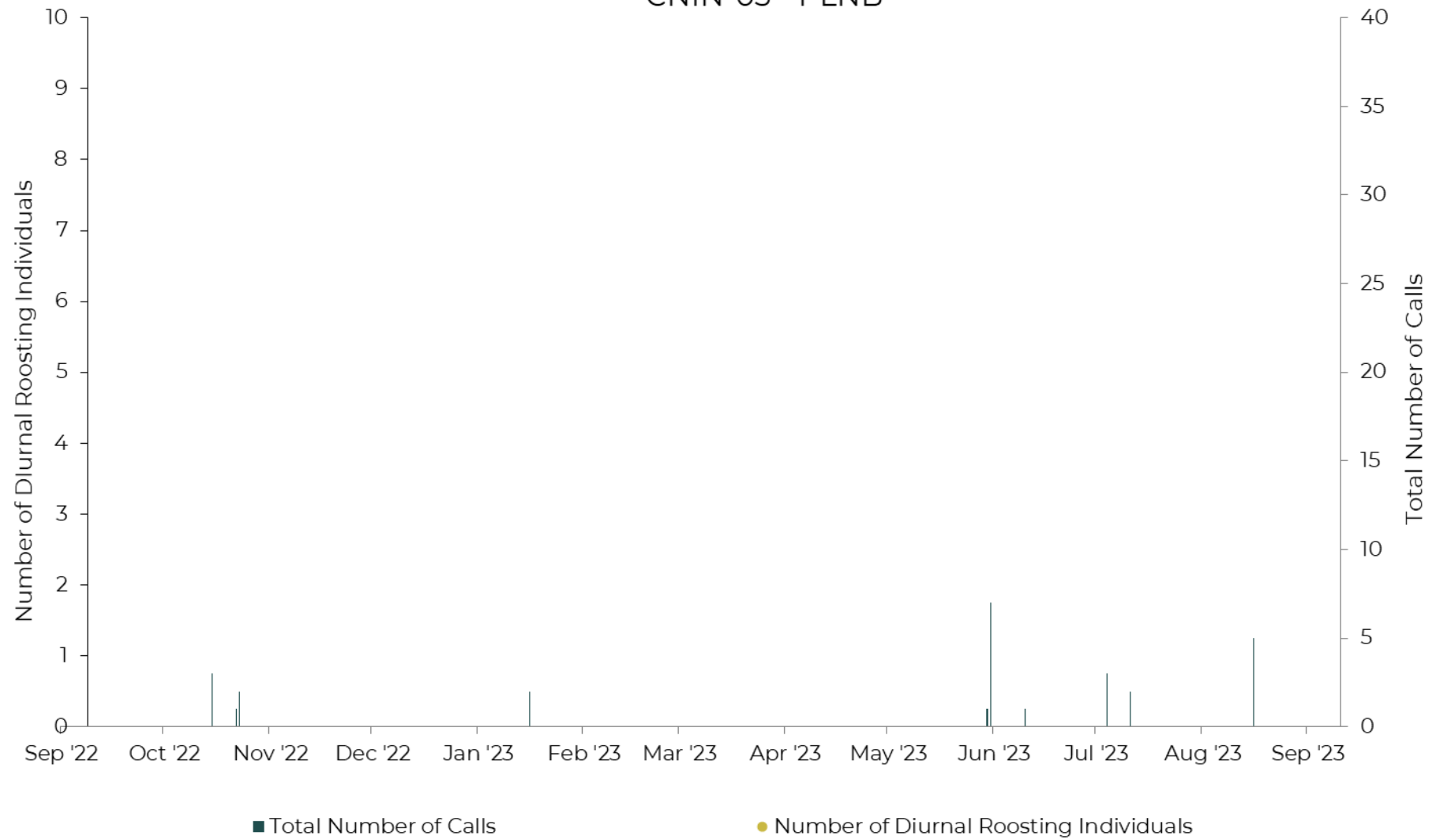




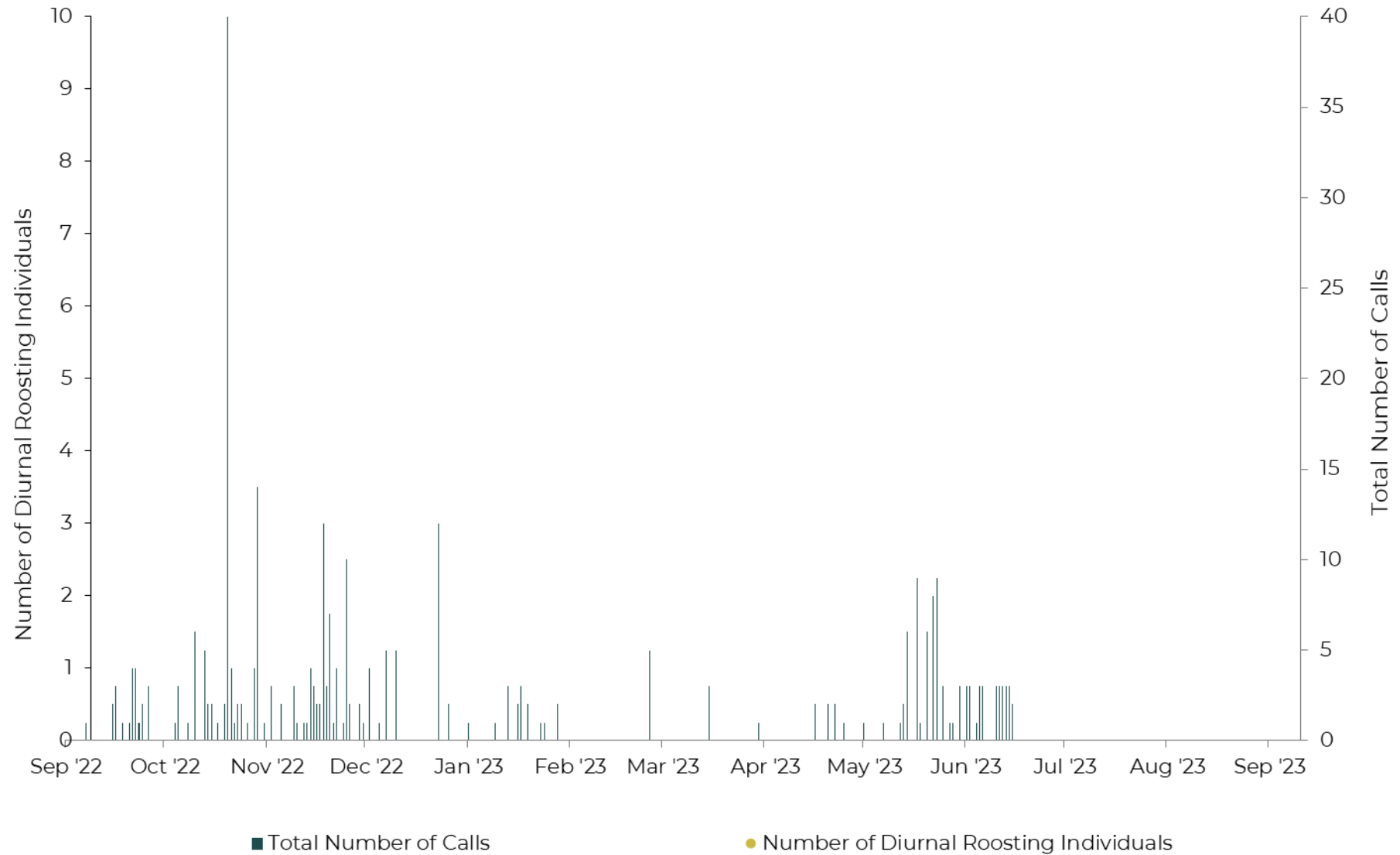
CCAT-06 - PLNB

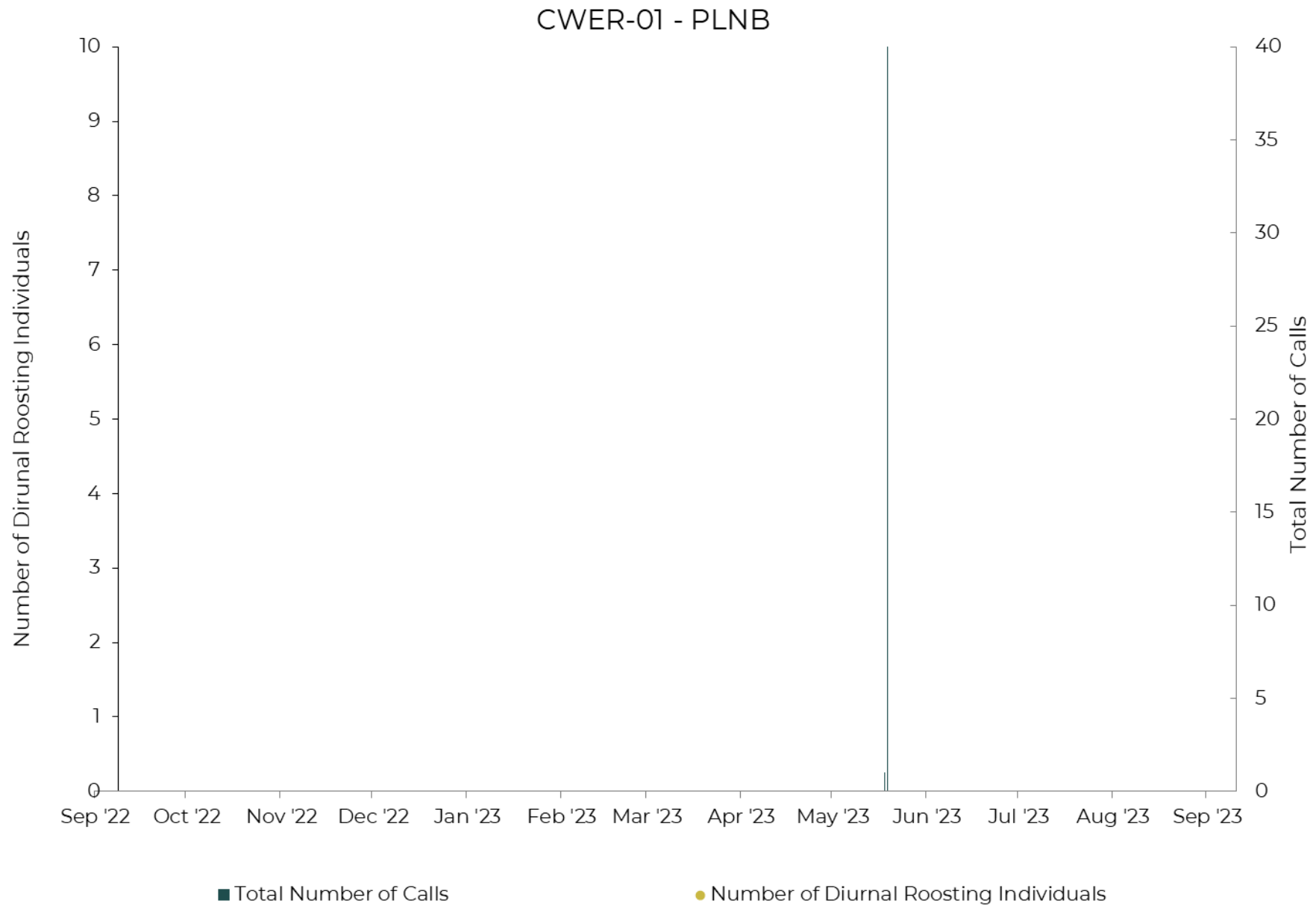


CNIN-03 - PLNB



CNIN-09 - PLNB





Appendix L: Cave Monitoring

Appendix Table 12: Monitoring cave distance to mining

Area	Cave ID	Category	Distance to Disturbance Area (km)	
			2023	2022
Western Ridge	CWER-01	Category 2	5.83	5.81
	CWER-03	Category 2	0.52	0.53
	CWER-04	Category 4	0.47	0.47
	CWER-05	Category 4	8.17	8.19
	CWER-07	Category 4	5.89	5.90
	CWER-09	Category 4	6.72	6.72
	CWER-10	Category 3	1.05	1.07
	CWER-14	Category 4	1.95	1.99
	CWER-16	Category 3	3.53	3.55
	CWER-17	Category 3	6.62	6.64
	CWER-20	Category 4	2.94	2.94
Jimblebar/ Ninga	CJIM-01	Category 4	2.64	2.63
	CJIM-03	Category 2	2.67	2.67
	CJIM-04	Category 4	0.64	0.64
	CJIM-05	Category 4	1.49	1.50
	CJIM-06	Category 4	0.37	0.38
	CJIM-07	Category 4	0.32	0.33
	CJIM-08	Category 4	0.34	0.34
	CJIM-09	Category 3	0.53	0.52
	CJIM-14	Category 3	0.67	0.67
	CJIM-15	Category 4	0.96	0.95
	CJIM-16	Category 3	3.58	3.58
	CJIM-17	Category 4	0.96	0.95
	CJIM-18	Category 4	1.35	1.34
	CJIM-20	Category 4	1.32	1.32
	CNIN-01	Category 3	4.78	4.78
	CNIN-02	Category 3	2.44	2.44
	CNIN-03	Category 2	3.23	3.24
	CNIN-09	Category 3	8.02	8.02
	CNIN-13	Category 3	8.53	8.51
Cathedral Gorge	CCAT-01	Category 3	1.66	1.69
	CCAT-02	Category 3	2.05	2.09
	CCAT-06	Category 3	5.37	5.37
	CCAT-09	Category 3	7.06	7.06

Area	Cave ID	Category	Distance to Disturbance Area (km)	
			2023	2022
	CCAT-10	Category 4	6.85	6.84
	CCAT-13	Category 3	1.92	1.74
	CCAT-14	Category 4	6.42	6.43
	CCAT-17	Category 4	5.20	5.19
	CCAT-21	Category 3	8.23	8.23
	CHST-08	Category 4	3.68	3.68

Note: **bolded** refers to caves where ghost bats were recorded via primary or secondary evidence

CJIM-03					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.40726979	120.2034153	PP02	-23.40791531	120.2031226
2022			2021		
2023			2022		

CJIM-04					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.35733798	120.16293	PP02	-23.35726934	120.1629729
2022			-	No Photo Available	
2023			2023		

CJIM-05					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.34973264	120.1363144	PP02	-23.34999951	120.1368039
2022			2022		
2023			2023		

CJIM-06					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.35955843	120.1637568	PP02	-23.35961845	120.1642191
2022			2022		
2023			2023		

CJIM-07					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.36006266	120.1634107	PP02	-23.36007491	120.16436
2022			2022		
2023			2023		

CJIM-08					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.36031126	120.1628751	PP02	-23.36009085	120.1643527
2022			January 2023		
2023			September 2023		

CJIM-09					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.36351998	120.1408429	PP02	-23.36376336	120.1407204
2022			2022		
2023			2023		

CJIM-14					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.35986531	120.1483502	PP02	-23.35891329	120.1490331
2022			2022		
2023			2023		

CJIM-15					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.35036243	120.1169844	PP02	-23.35015188	120.1172144
2022			2022		
2023			2023		

CJIM-16					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.41652276	120.2040868	PP02	-23.41669321	120.2051111
2022			2022		
2023			2023		

CJIM-17					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.35036243	120.1169844	PP02	-23.35015188	120.1172144
2022			2022		
2023			2023		

CJIM-18					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.35300314	120.1396471	PP02	-23.35444678	120.1397225
2022			2022		
2023			2023		

CJIM-20					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.409694	120.112097	PP02	-23.40939329	120.1113645
2022			2022		
2023			2023		

CNIN-01					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.32311911	119.9815594	PP02	-23.32327366	119.981605
2022			2022		
2023			2023		

CNIN-02					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.32013603	120.0062565	PP02	-23.32032292	120.0061525
2022			April 2023		
2023			September 2023		

CNIN-03					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.407915	120.203123	PP02	-23.407270	120.203415
2022			2022		
2023			2023		

CNIN-09					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.31568278	119.9320594	PP02	-23.3166419	119.9315544
2022			2022		
2023			2023		

CNIN-13					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.31829239	119.9452295	PP02	-23.31823702	119.9451412
2022			2022		
2023			2023		

CWER-01					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.41262954	119.5835373	PP02	-23.41296459	119.5859902
2022			2022		
2023			2023		

CWER-03					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.397000	119.660730	PP02	-23.395971	119.660693
February 2023			February 2023		
September 2023			September 2023		

CWER-04					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.39673826	119.6603903	PP02	-23.39694965	119.6606367
2022			June 2023		
2023			September 2023		

CWER-05					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.41214991	119.5567048	PP02	-23.41215082	119.5566976
2022			2022		
2023			2023		

CWER-07					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.40980899	119.5806452	PP02	-23.40886728	119.5805933
June 2022			2022		
September 2022			2023		

CWER-09					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.41042492	119.5715586	PP02	-23.41043059	119.5715569
2022			2022		
2023			2023		

CWER-10					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.40307568	119.660343	PP02	-23.4034614	119.6603385
2022			February 2023		
2023			September 2023		

CWER-14					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.4114932	119.6473538	PP02	-23.41192669	119.647461
2022			2022		
2023			2023		

CWER-16					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.405139	119.605518		-23.406040	119.605242
February 2023			February 2023		
-	No photo available.		Sept 2023		

CWER-17					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.408614	119.571596	PP02	-23.408828	119.571901
2022			2022		
2023			2023		

CWER-20					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.3984321	119.6070936	PP02	-23.39892174	119.6073054
2022			2022		
2023			2023		

CCAT-01					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.28795861	119.7452386	PP02	-23.28790732	119.7456776
2022			2022		
2023			2023		

CCAT-02					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.28356111	119.7486408	PP02	-23.2833917	119.7482158
2022			2022		
2023			2023		

CCAT-06					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.28212755	119.7024158	PP02	-23.28201545	119.7028309
2022			2022		
2023			2023		

CCAT-09					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.27650166	119.6322586	PP02	-23.27645177	119.6321218
2022			2022		
2023			2023		

CCAT-14					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.27536808	119.6615294	PP02	-23.27479869	119.6617961
2022			2022		
2023			2023		

CCAT-17					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.28001021	119.7055245	PP02	-23.27963601	119.7050773
2022			2022		
2023			2023		

CHST-08					
Photo Point ID	Location		Photo Point ID	Location	
	Latitude	Longitude		Latitude	Longitude
PP01	-23.29687871	119.7141425	PP02	-23.29681774	119.7133522
2022			2022		
2023			2023		