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Activity budgets, responses to disturbance and novel behaviours in captive mountain chicken frogs *Leptodactylus fallax*

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Mountain chicken frogs *Leptodactylus fallax* are assessed as Critically Endangered on the IUCN Red List due to threats including chytridiomycosis and habitat loss. Ex-situ populations underpin species survival, but captive management is hampered by incomplete species knowledge, including its behavioural biology. In sixteen adult frogs, we investigated enclosure usage, nocturnal activity budgets, and behavioural responses to varying levels of husbandry-related disturbance through instantaneous scan sampling of camera trap footage over forty-two consecutive nights. Enclosure usage was quantified through the application of modified Spread of Participation Indices (mSPI). We present the first detailed activity budgets published for this species and found that broad activity patterns of captive animals corresponded well with their wild conspecifics; taking refuge during the day and emerging at dusk into exposed areas of the enclosure. Some behaviours, especially hunting and bathing, were partially phased throughout the nocturnal period. Enclosure use was not even, with disproportionately large amounts of time spent using nest boxes and deep leaf litter, even at night, and the converse in thin leaf litter and elevated perches. Principal Components Analysis (PCA) identified a bold-shy behavioural grouping (movement and resting opposed to refuge behaviours) that was consistent across husbandry-related disturbance conditions. Randomisation analyses showed that disturbance significantly affected multiple measured behaviour, especially feeding, social interactions and vocalisation; the impact was associated with the degree of disturbance. Novel behaviours were also documented, which are thought to be related to courtship. Our data provide insights into the behaviour of mountain chicken frogs and highlight potential trade-offs between enclosure maintenance, veterinary intervention, and animal welfare.

Keywords: Amphibians, behaviour, mSPI, disturbance, welfare, Zoo

INTRODUCTION

Amphibians are undergoing a global mass extinction; 42 % of amphibian species are at risk of extinction, mediated principally by habitat destruction and emerging infectious diseases (Scheele et al., 2019). The mountain chicken frog *Leptodactylus fallax* was once widely distributed among the Eastern Caribbean islands. The introduction of the fungal pathogen *Batrachochytrium dendrobatidis* (Bd), alien predatory species, habitat loss and over-harvesting caused significant declines and the species is now restricted to the islands of Dominica and Montserrat (Adams et al., 2014). The species is assessed as Critically Endangered with fewer than 200 individuals remaining in the wild on Dominica and being likely extinct on Montserrat (IUCN SSC ASG, 2017). Mountain chickens are nocturnal and terrestrial, and one of the largest extant frog species (Jameson et al., 2019) and exhibit a unique mode of reproduction (Gibson & Buley, 2004). Males form territories around terrestrial burrows which they guard against rival males and to which they attract females through advertisement calling. Within the burrow, the frogs create a foam nest in which larvae develop into metamorphosis, fed with infertile

eggs by the mother (see Gibson & Buley, 2004). They have cultural and economic value to local communities (Nicholson et al., 2020). These factors have fuelled an international collaborative effort to save this species in the wild (Adams et al., 2014; Jameson et al., 2019).

Creating a sustainable captive population is essential for the long-term survival of this species (Adams et al., 2014; IUCN SSC ASG, 2017). Multiple captive populations were established, between 1998 and 2011, at Metro Toronto, Jersey and ZSL London Zoos and in a breeding centre in Dominica, as safety net populations and for conservation education. These populations did not ultimately contribute to conservation translocations (Gibson & Buley, 2004; Cunningham et al., 2008; Tapley et al., 2014; Jameson et al., 2019). In 2009, in response to disease mediated population collapses, 50 wild mountain chickens were collected from Montserrat and were distributed to multiple European institutions where breeding has produced frogs for translocations, and founded a separately managed non biosecure population for staff training and public education (Hudson et al., 2016).

Although successful captive breeding has led to an increase in this captive population size (Jameson et al.,

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Figure 1. Mountain chicken enclosure at ZSL London Zoo, illustrating enclosure layout and overlapping camera angles providing coverage of the entire enclosure.

2019), managing mountain chickens in captivity has proven problematic, mainly due to the highly specific conditions required by this species in captivity (Tapley et al., 2015; Donaldson, 2019; Jameson et al., 2019). The physiological requirements of captive mountain chickens are becoming better understood (Fitzgerald et al., 2007; Dierenfeld et al., 2008; King et al., 2011; Jaffe et al., 2015; Tapley et al., 2015; Jayson et al., 2018; 2018b; Croci et al., 2019; Donaldson, 2019; Jameson et al., 2019; Ashpole et al., 2021; Michaels et al., 2021; White et al., 2021). Although very basic information on habitat use and activity patterns is known from wild populations (Schwartz & Henderson, 1991; Daltry, 2002; see Jameson et al., 2019), detailed information on activity budgets and patterns is lacking for both wild and captive mountain chickens. Behavioural data are important to inform the management of wild and captive animals (Estevez & Christman, 2006; Ross et al., 2009). We investigated baseline enclosure usage and nocturnal activity patterns in captive mountain chicken frogs and assessed whether these were affected by husbandry-related disturbance.

MATERIALS & METHODS

Ethics statement

This study was reviewed by the Zoological Society of London (ZSL Projects Database ref. number WHB7) and, as all data were collected from observations of normal husbandry practice, it was not deemed necessary to undertake full ethical review, nor was the study subject to the Animals (Scientific Procedures) Act 1986. The work is in line with the BHS Ethics Policy.

Study subjects

We observed sixteen adult mountain chicken frogs housed at ZSL London Zoo; eight captive-bred males, seven captive-bred females (ages ranging from 1 year 6 months – 1 year 8 months at time of study) and one wild-collected female (17 years 1 month in captivity, estimated age c. 23 years, at time of study). Frogs were housed in four groups of two females and two males, which were the experimental units in this study.

Enclosure Design and husbandry

Frogs were housed in 2x2 m dens with bark chip substrate, aligned back-to-back with a brick wall and wire mesh separating them (Fig. 1). Furnishings consisted of leaf litter, 2 x large artificial palm leaves, 2 x clay-lined nest boxes with tubes for entrance. Detailed husbandry information is given by Michaels et al. (2021). Animals were fed three times a week, but food insects were almost always present in dens between feedings, as frogs do not capture all items immediately. In order to collect accurate temperature data, four data loggers (EasyLog EL-USB-1-LCD, Lascar Electronics, Wiltshire, UK) were evenly distributed across each enclosure (refer to Fig. 2) and recorded temperature every five minutes throughout the study. Mean temperature across enclosures was 24.26 °C with a range from 22.61–25.39 °C.

Observations and disturbance events

Frogs were observed between 11 April (night 0) until and including 23 May 2021 (night 42), during which period the frogs were in their breeding season. Observations occurred between the hours of 1700 and 0830 the next day. Lights (full spectrum specialist lighting arrays - see Michaels et al., 2021 for details) within the dens were on until 1800 after which they remained switched off until 0745 the following morning.

Observations were recorded using two time-lapse infra-red based night vision video cameras (Crenova Trail Camera 186 PH760) per den, which captured the entire den in combined field of view. Therefore, frogs were only out of sight when in refuges (nest boxes and other refugia) and so were coded as showing refuge behaviour (see below; Table 1).

Review of preliminary diurnal footage showed almost no observations of visible frogs and so data collection was limited to the nocturnal period, including a brief period of light at the start and end of each recording. As there was no internet or other communications access in the frog facility, all data was stored locally and omission of diurnal recording substantially reduced required incursions into the facility to change storage cards, as well as reducing personnel interactions during the COVID-19 pandemic.

Table 1. Ethogram used for coding mountain chicken frog behaviour

Behaviour	Definition
Refuge use	Stable position - partially visible or not visible at all. Using furnishing/substrate to conceal itself. Note: in this study if the animal is out of sight, we are determining it as a 'refuge use' behaviour.
Resting	50 % or more of the body is visible and stationary.
Movement	Can be observed either crawling (whole body movement across the enclosure in a slow manner with the body near to the ground), hopping (fast process whole body movement jumping across the enclosure using their hind limbs) or climbing (whole body movement with the intention of ascending). Climbing usually occurs on top of the window ledge of each enclosure, ascending up the wired mesh. If a frog is stationary on the mesh, this too is classified as movement.
Hunting	Actively stalking prey (for an example slow body movement towards prey item with eyes orientated towards it) or lunging on prey and consuming.
Intra-specific social interaction	Any social interactions observed such as following a conspecific to a different area of the enclosure or moving towards a stationary conspecific in close proximity (within three body lengths), submissive or dismissive interaction, an act of dominance such as chasing or fighting.
Vocalisation	Single, or multiple continuous calls.
Bathing	Bathing in water bath either alone or with a conspecific
Other	Any other notable behaviours that have not been categorised as focal behaviours.

Cameras were programmed to record continuous four-minute video clips with an interval time of fifteen minutes; these video clips were later analysed by the observer.

An ethogram (Table 1) was developed based on a subset of the footage. Individual frogs were not reliably identifiable on camera trap footage, so nocturnal observations were conducted using instantaneous scan sampling for each den. The number of frogs performing each behaviour was counted in each den every five seconds from each four-minute video clip and data were recorded in Microsoft Excel for Windows 10. Ad-hoc descriptive observations of behaviours of note were also made contemporaneously. Total counts of each behaviour per den per night (i.e. for the entire nocturnal period) were calculated and used for analysis.

Frogs were exposed to different intensities of disturbance due to routine husbandry procedures during the observation period. Data for all dens collected prior to night 29 were designated as baseline disturbance intensity (disturbance intensity A), characterised by standard husbandry with no recent or current disturbance.

Dens 2 and 4 were simultaneously subject to a full substrate change. This involved briefly capturing and containing frogs in their nest boxes, while all furnishings were removed from the den and cleaned, and the bark mulch substrate was removed and replaced with fresh material. This took approximately 60 minutes per den between containment of frogs and release from the nest box, and took place between 0900 and 1100.

In the morning prior to night 29, three frogs in den 2 (disturbance intensity C) and one frog in den 4 (disturbance intensity D) underwent invasive health checks. An invasive health check is part of recommended health screening for the captive population (Jameson et al., 2019) and consists of catching frogs by hand, transport to veterinary facilities, isoflurane anaesthesia, blood sampling, radiography and ultrasonography,

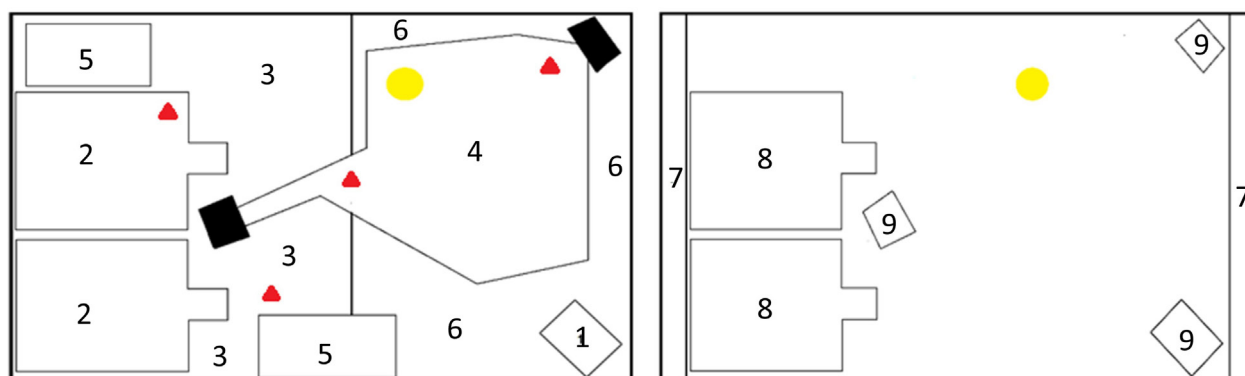


Figure 2. Diagrams illustrating resource zones within mountain chicken enclosures. Zone numbers correspond to Table 2 and are defined as 1: Large 'tipped over' plant pot lined with leaf litter; 2: Enclosed, nest box lined with modelling clay; 3: Thin layer of leaf litter; 4: Large palm leaves with a thick layer of leaf litter underneath; 5: Water dish; 6: Thick layer of leaf litter; 7: Window ledge; 8: Thin layer of leaf litter on top of the nest box; 9: The top surface of the 'tipped over' plant pot. The left-hand panel shows floor-level resources, the right represents the higher tiers, red triangles show the location of the 4 data loggers, yellow circles indicate the location of basking lamps and black polygons show positions of plant pots filled with substrate that do not provide a floor-level refuge but do for elevated resource zones (zone 9, right-hand panel).

Table 2. Resource zones into which enclosures were divided for mSPI analysis

Zone	Description of each zone	Biological relevance	Percentage cover for each enclosure (%)			
			1	2	3	4
1	Large 'tipped over' plant pot lined with leaf litter	Empty plant pot, filled with leaf litter acts as a retreat within the warm zone	3.7	3.4	3.9	3.4
2	Enclosed, nest box lined with modelling clay	Nest building, refuge, within the cool zone	9.1	9.0	8.8	9.1
3	Thin layer of leaf Litter	Leaf litter unsuitable for hiding as too shallow. Includes a temperature gradient from warm to cool zones across the enclosure	30.4	29.4	29.9	30.7
4	Large palm leaves with a thick layer of leaf litter underneath	Artificial plants and leaf litter provide effective refuges; incorporates a temperature gradient across enclosure from warm to ambient zones	16.1	15.3	15.9	15.9
5	Water dish	2 x large plastic tray, filled with fresh water daily. Ambient and cool temperature zone.	5.8	5.6	5.2	6.0
6	Thick layer of leaf litter	Deep leaf litter layer suitable for hiding with temperature gradient from warm to ambient zones.	12.2	11.7	11.7	12.6
7	Window ledge	An 80 mm ledge positioned 50 cm off the ground. When on top of the window ledge, frogs can see into other enclosures housing mountain chicken frogs. Fully exposed so cannot use zone as refuge.	5.8	9.3	9.8	5.4
8	Thin layer of leaf litter on top of the nest box	Fully exposed, cannot use this zone for refuge. Elevated so frogs can see rest of enclosure, but not high enough to see into neighbouring enclosures. Cool zone of enclosure.	11.1	11.0	10.8	11.1
9	The top surface of the 'tipped over' plant pot	Fully exposed, cannot use this zone as refuge. Elevated so frogs can see rest of enclosure, but not high enough to see into neighbouring enclosures. Warm zone of enclosure.	5.8	5.3	4.9	5.8

followed by recovery and return to the den. The process took approximately 90 minutes from capture to release for all involved frogs together.

Therefore, dens 2 and 4 experienced direct disturbance; they were subject to a full substrate change as well as some individuals undergoing capture and health checks. Dens 1 and 3 experienced no direct disturbance, but both were subject to indirect disturbance from dens 2 and 4 (see above) due to the prolonged presence of humans and vibrations from moving refugia and substrates (disturbance intensity B).

These arrangements resulted in four disturbance intensities: A - pre-disturbance baseline, B - indirect disturbance - substrate change, C - high intensity direct disturbance - 3 frogs removed from den 2 for health checks, and D - low intensity direct disturbance - 1 frog removed from den 4 for health checks.

Enclosure usage

To evaluate enclosure usage, we used Plowman's (2003) modified Spread of Participation Index (mSPI). This equation produces values ranging from 0–1; a value of 0 indicates that frogs use all zones equally whereas a value of 1 suggests that frogs favour only one zone of the enclosure.

$$\text{mSPI} = \frac{\sum |f_o - f_e|}{2(N - f_{e \min})}$$

f_o = observed frequency of observations.

f_e = expected frequency of observations in a zone, calculated as the total number of observations multiplied by the proportion of the enclosure represented by the zone.

$|f_o - f_e|$ = absolute value of the difference between f_o and f_e

\sum = sum of all zones

N = total number of observations in all zones

$f_{e \min}$ = expected frequency of observations in the smallest zone

Dens were zoned based on biological relevance, i.e. based on qualitatively different microhabitats and relevance to different behaviours (Fig. 2.; Table 2). At the same time as behavioural monitoring, the number of frogs in each zone was recorded; the total number of observations in each zone was recorded for each den for each night. For refuge zones (see Table 2; zones 1, 2, 3, 4 & 6), video footage was reviewed to confirm frogs entering and moving between refuges to ensure that frogs were counted in the correct refuge type. If location could not be confirmed, data were omitted. If a

frog was partly in two zones at the same time, the zone containing the majority of the frog was recorded; if a frog inhabited two zones equally, the zone containing the head was recorded; if the head was positioned where the boundary was down the midline between each zone, the zone that the frogs' eyes were oriented towards was recorded. mSPI was calculated per night per den.

Statistical analyses

Statistical analysis was conducted using R version 4.1.1 in RStudio Version 1.4.17 for Windows (R Core Team, 2021). An Alpha of 0.05 was used throughout the study unless otherwise indicated.

Activity budget phasing

Total counts of each behaviour exhibited by all frogs during the undisturbed phase of the study were calculated. These data were partitioned between three segments of the nocturnal period (1700–2159, 2200–0259, 0300–0830) to assess phasing inactivity budgets across the night. Behavioural counts split across time period were assessed visually as interdependence between observations (as they were from the same individuals) prevented formal analysis without pseudoreplication.

Behavioural changes in response to disturbance

In order to minimise the number of behavioural variables analysed, following Martin & Réale (2008), we used a Principal Components Analysis using FactoMineR (Lê et al., 2008) and Factoextra (Kassambara & Mundt, 2020) to identify behavioural groupings that were consistent across both before and after disturbance conditions following. Data were standardised with mean 0 and standard deviation 1 prior to analysis so that the covariances were robustly comparable. Eigenvalues >1 were used to select dimensions retained, and relative weightings for each behaviour in each dimension were used to identify the dimensions primarily representing those behaviours. Hunting was not included in this process as it was identified as a variable of specific interest given keeper anecdote that food intake appeared to reduce after disturbance (see Discussion). A dimension comprising movement, refuge and resting was identified as present before and after disturbance, and the co-ordinates associated with this dimension were used for analysis, along with raw data for remaining behaviours.

We compared baseline (A) to disturbed (B, C or D) data for each frog enclosure. We used the shuffle function within the Mosaic package (Pruim et al., 2017) to run randomisation analyses with 10,000 iterations in order to test for an effect of disturbance on behavioural counts and mSPI. Randomisation is a valid strategy for analysing small- and single-n samples and is useful when working with small sample sizes in zoo contexts (Dugard et al., 2012). The residual (i.e. the difference) between the means of each before-after disturbance pair was used as a test statistic. The data were then shuffled randomly 10,000 times and a new test statistic

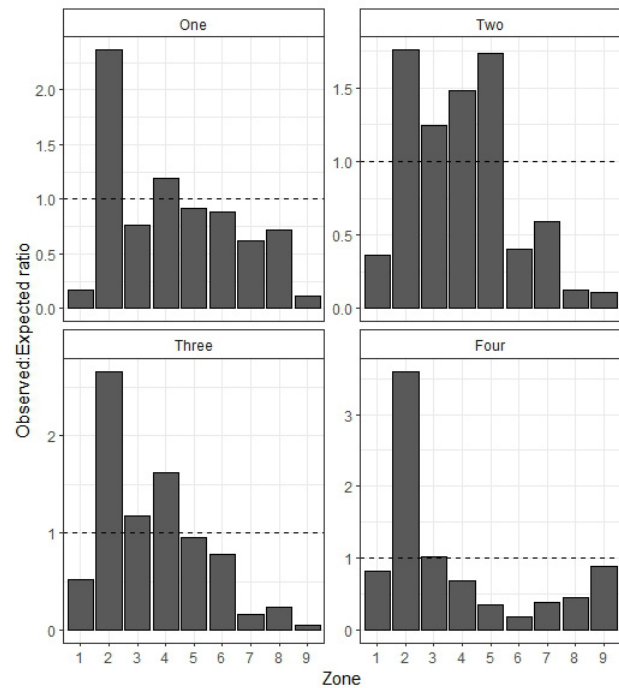


Figure 3. Ratios of observed:expected (as calculated via modified spread of participation indices) counts of frogs in each den (one–four) occupying each enclosure zone under pre-disturbance baseline conditions. The dashed line indicates where observed and expected counts did not differ.

calculated; a two-tailed p-value was derived for each comparison from the overlap of simulated test statistics with the observed test statistic. Bonferroni corrections were applied to adjust for multiple comparisons.

RESULTS

Baseline enclosure usage, behavioural phasing and activity budget

Mean (SD) mSPI, pre-disturbance, for dens 1–4 were 0.35 (0.084), 0.38 (0.080), 0.38 (0.09), 0.48 (0.148), respectively. Figure 3 shows total observed zone usage compared with expected values under perfectly even zone usage; patterns between dens were similar with over-use of zone 2 and under- or quasi-expected use of other zones.

Figure 4 shows mountain chicken frog nocturnal activity prior to disturbance; all frogs spent the majority of their time either resting (51.7 %) or taking refuge (38.6 %). Frogs spent 5.4 % of their time using the water baths. Less than 10 % of time was spent in social interactions, movement and hunting, combined. Frogs remained active throughout the night, but behaviours do not appear to have been equally distributed across the three time periods for some behaviours, with animals tending to engage in active behaviours (movement, hunting, vocalisation) earlier in the night, and bathing behaviour later in the night, while the other behaviours are more evenly distributed (Fig. 5). Note that the final time period is slightly longer (30 minutes) than the first and second periods.

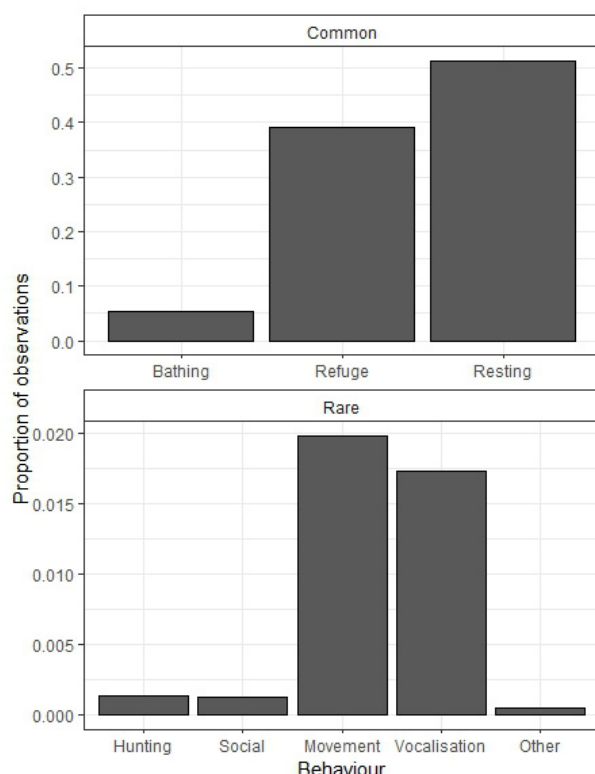


Figure 4. Proportion of observations of each behaviour type under pre-disturbance conditions aggregated across all dens and all nights; the top pane contains common behaviours, the lower pane rare behaviours.

Effects of disturbance from substrate change and invasive health checks

We retained only the dimension relating to movement, resting and refuge. The PCA identified a grouping between movement, resting and refuge, with movement and resting both negatively correlated with refuge (average co-ordinates 0.62, 0.799 and -0.89, respectively; see Table 4). Randomisation analysis showed that disturbance had a significant effect on several behaviours.

Most strikingly affected was hunting behaviour, which reduced significantly under all disturbance intensities. The behavioural grouping comprising movement, resting and refuge showed significant reduction (i.e. reduction in movement and resting and increase in refuge) after disturbance intensity C (catch-up of three out of four frogs) in den 2, but not in other dens (i.e. not in dens 1 or 3 under intensity B, or in den 4 under intensity D). Social interaction significantly decreased in both dens 2 and 4 (following catch-up of three and one of four frogs, respectively); vocalisation also decreased in den 2. (Fig. 6; see Table 3 for statistical outcomes).

Other behaviour

Two novel behaviours for mountain chicken frogs were observed on camera trap footage. These are described in Table 4. Behaviour 1 was coded as a social interaction, and Behaviour 2 as an other behaviour.

DISCUSSION

Review of preliminary footage showed that mountain chickens were almost entirely out of sight when lights were on and emerged from refugia when lights were switched off. These findings correspond well with previous research on their wild conspecifics; wild frogs were observed to retreat during the daytime and move into exposed, open areas at dusk (Jameson et al., 2019; Daltry, 2002). Temperatures in enclosures reflected natural temperatures of mountain chicken frog microhabitat in Dominica (20–25 °C) and Montserrat (21–24 °C), as did the photoperiods (Jameson et al., 2019), suggesting that frogs in this study were exposed to environmental cues broadly similar to those experienced by free living frogs.

Our data show that some behaviours appear to be partially phased throughout the night (Fig. 5). Although all behaviours were observed in all parts of the nocturnal period, frogs tend to emerge soon after dark and engage in hunting and movement behaviours, but bathe

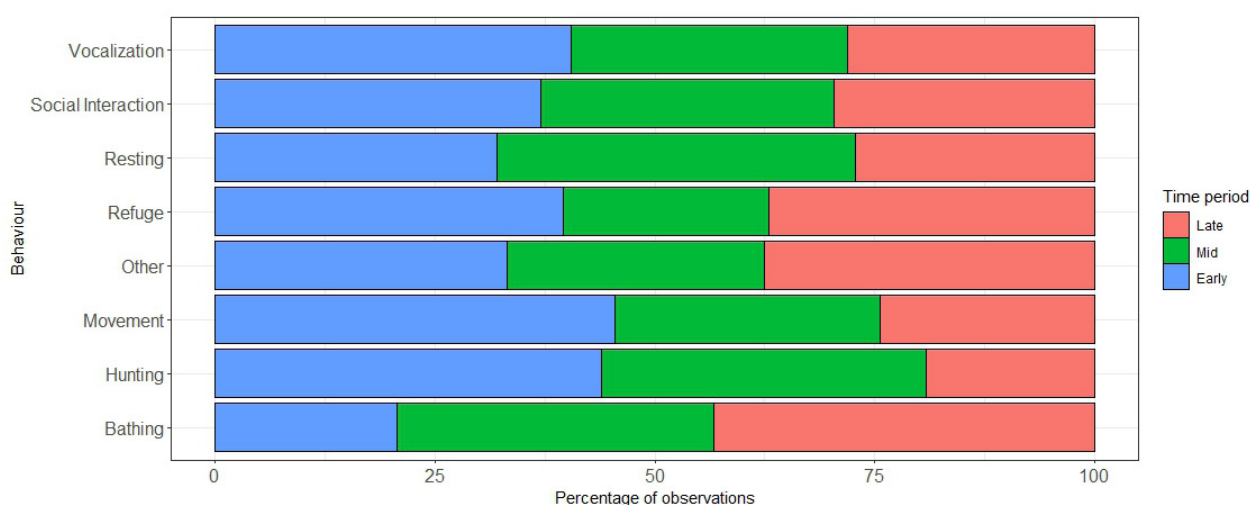
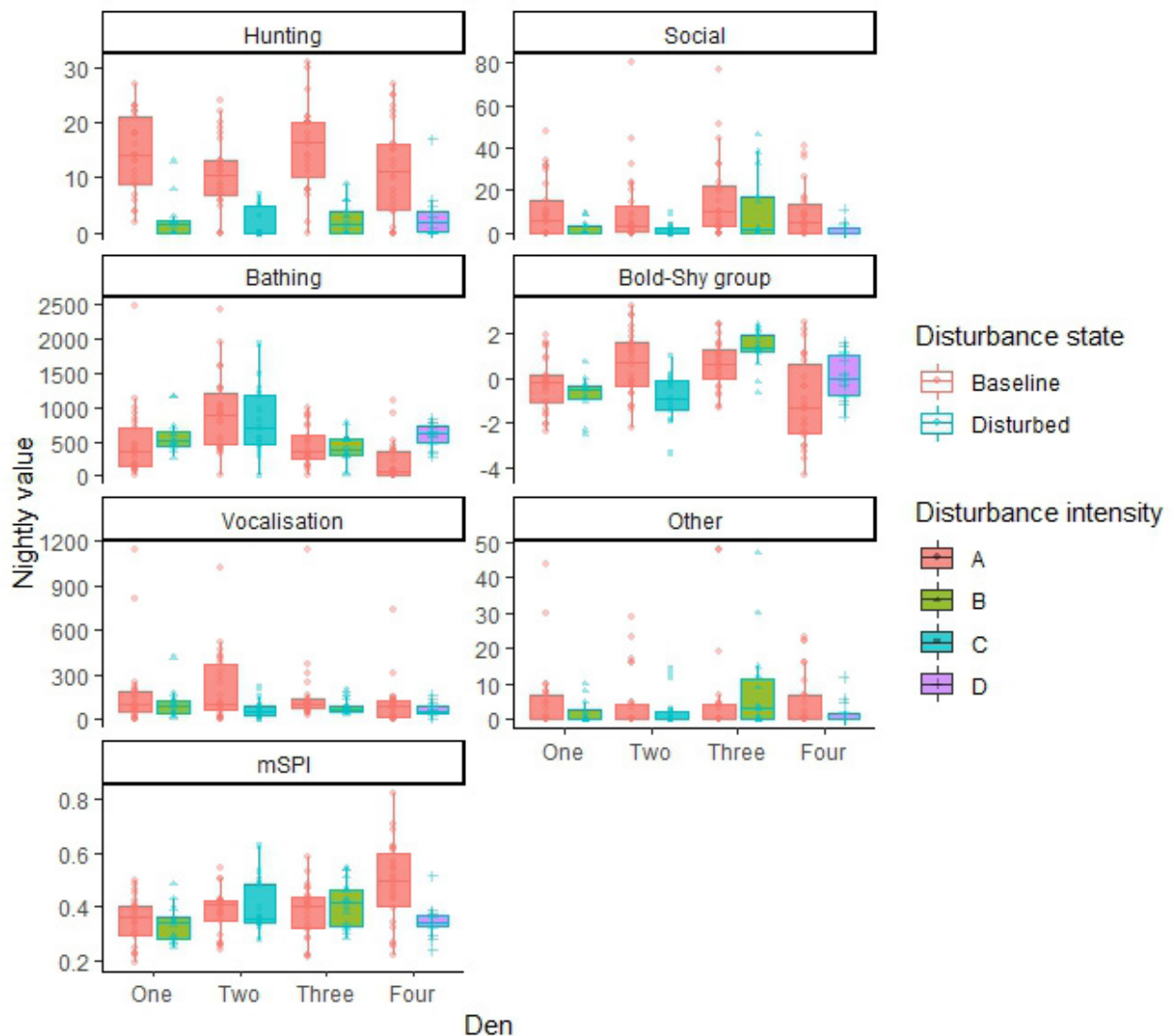


Figure 5. Total proportion of observations of each behaviour, expressed as a percentage of all observations of that behaviour, split between the early (blue), mid (green) and late (pink) thirds of the nocturnal period.

Table 3. Statistical outcomes (p-values) of randomisation tests, to three decimal places, comparing behaviours before and after disturbance in each of four dens. Bonferroni corrections were applied; significant values after corrections are in bold

Den	Treatment comparison	P value (2 tailed)						mSPI	Bold/shy grouping
		Bathing	Hunting	Social interaction	Vocalisation	Other			
1	A vs B	0.500	<0.001	0.018	0.417	0.180	0.732	0.075	
2	A vs C	0.597	<0.001	0.010	0.009	0.34	0.440	<0.0001	
3	A vs B	0.583	<0.001	0.364	0.067	0.4	0.391	0.020	
4	A vs D	<0.001	<0.001	0.004	0.444	0.060	<0.001	0.052	

**Figure 6.** Nightly counts of each mountain chicken frog behaviour and mean mSPI, split by disturbance state and intensity, and by den. Boxplots show median, interquartile range and outliers (to which randomisation analysis is robust) identified as $> 1.5 \times \text{IQR}$ above Q3 or below Q1. Refer to Table 3 for relevant statistical comparisons, which can only be made within dens.

primarily in the final part of the night. Other behaviours are more evenly distributed. Frogs absorb water to assist with digestion of food, with the mass of water absorbed roughly equalling food intake (Hillman et al., 2009), so frogs may bathe more later in the night to absorb water after feeding earlier in the night. The inability to see inside nest boxes and refugia, where frogs spent around 40 % of their time, is a limiting factor to fully elucidating activity

budgets, as we do not know whether these spaces conceal additional social interactions or other behaviours.

Enclosure usage under undisturbed conditions measured through mSPI was slightly uneven; adjustments to the design of dens could expand the proportions of dens covered by favoured resource zones. Zone 2 (nest box) was particularly over-used, whereas zones 6 and 8 (thin leaf litter zones), and 7 and 9 (elevated zones) were particularly

Table 4. Co-ordinates and contributions associated with the PCA dimension grouping movement, refuge and resting behaviours, with a comparison of outcomes from all data, and data from before and after disturbance. As consistency across conditions was established for this grouping, co-ordinates from the dimension across all data could be used. Other behavioural groupings did not demonstrate consistency across conditions and so raw counts of individual behaviours were used for randomisation analysis.

Behaviour	All data			Before disturbance			After disturbance		
	Average co-ordinates	Average contributions	Eigenvalue	Average co-ordinates	Average contributions	Eigenvalue	Average co-ordinates	Average contributions	Eigenvalue
Movement	0.62	17.01		0.67	18.424		0.60063	14.68	
Refuge	-0.89	35.47	2.46	-0.92	34.80	2.43	-0.84662	29.16	2.46
Resting	0.79	27.98		0.79	25.55		0.79	25.55	

under-used (Fig. 3). The over-usage of the nest box during the nocturnal period highlights the importance of this resource. Through anecdotal observation of this species in captivity during the day, frogs typically use the nest box as a diurnal retreat (Tapley, Servini & Michaels, pers. obs.) and our data show that this pattern is maintained at night. This is particularly important for territoriality as nesting-sites form the core resource in, especially male, frog territories (see Jameson et al., 2019). As such this over-use is unlikely to be a welfare concern, as it reflects the tendency of mountain chicken frogs to live in burrows and other small subterranean spaces (Jameson et al., 2019; Daltry, 2002). However, it does indicate that adequate nest box space should be provided for the number of frogs present in an enclosure and that other refugia may not be equivalent as a hide. The under-use of thin leaf litter indicates the importance of deeper leaf litter zones (zone 4, which was not under-used) for mountain chickens. Our data therefore support the use of deeper leaf litter, under which frogs can be completely covered, across the enclosure to cater to frog behaviour; however, this must be balanced with the need for frogs to find food easily and before it has lost nutritional quality and to be exposed to UVB lighting (Jameson et al., 2019). The under-use of elevated positions is likely a result of these exposed zones being used primarily for behaviours associated with territory defence and mate attraction (Jameson et al., 2019). Although not used as frequently as expected by chance, frogs did regularly use these zones, despite being difficult to access. This highlights that mountain chickens are agile animals and that captive environments should provide opportunities for climbing, elevated resting and vocalisation. Frogs regularly climbed to more than 1.5 m above ground level on mesh walls and used perches at approximately 1 m height for vocalising from a resting position.






In alignment with other authors (see Jameson et al., 2019), we observed substantial bouts of combat between male frogs sometimes lasting more than four minutes, whereby animals attempted to flip one another using their nuptial spurs to grasp opponents while pushing against the floor with their hind legs. These fights appeared to be energetically expensive and support observations of scratches and other injuries in

male frogs thought to derive from nocturnal combat (Jameson et al., 2019).

We also made several novel observations of behaviour in mountain chicken frogs. These behaviours were included in broader behavioural categories, but specific description may be useful in managing this species in captivity and understanding its natural history in the field. In line with Daltry & Gray (1998) and Daltry (2002), we observed reproductive advertisement behaviour (primarily vocalisation) from males outside of the nest box entrance and in elevated, exposed positions. However, in a novel observation, on multiple occasions amplexus was observed outside the nest box in exposed, flat areas. Couples either separated or moved into nest boxes while still amplexant.

We observed previously undescribed social behaviours between males and females. In Behaviour 1 (Table 5), which was conserved in sequence between several observations, a female frog approaches a vocalising male near to a nest box entrance. The female's body posture is low and flat to the ground, usually calling softly, in which position she crawls very slowly forwards, attempting to move underneath the male. Once moving, female vocalisation stops. Meanwhile the male vocalises continuously, often in alternating synchrony with other males, reflecting wild observations of males alternating rather than overlapping their respective calls (Davis et al., 2000). When the female makes physical contact, with her snout touching the underside of the male's throat, the male stops vocalising and crawls away. The function of this behaviour is not clear, but given that it only occurred between females and males, and incorporated vocalisation from both sexes, the slow movement and exaggerated posture is likely linked to courtship. In Behaviour 2 (Table 5), male frogs moved their folded hind legs side to side with increasing frequency and then came to rest. Again, the function for this behaviour is not clear, but it is again likely linked to communication between individual frogs; sometimes frogs were not clearly associated with another frog when performing this behaviour. The exaggerated and repeated movements are almost certainly visible to more distant frogs within an enclosure since mountain chickens possess good night vision (see Jameson et al., 2019).

Table 5. Novel behaviours in mountain chicken frogs described from video footage from this study

Image	Description and/or timeline of behaviour
	Behaviour 1:
1. 	These images demonstrate a timeline of Behaviour 1, which appears to be part of courtship.
2. 	1. The male (left, image 1) was positioned near the entrance of the nest box in an exposed position and vocalised continuously throughout the night; alternately synchronised with a male in another enclosure; it appeared that the female (right, image 1) was attracted to his calling.
3. 	2. The female (right, image 2) positions her body flat to the ground then slowly attempted to crawl under the male (left) from the anterior end.
	3. The male immediately stopped vocalising and crawled away from the female (image 3).
	4. A few seconds later, he continued vocalising throughout the night.
	The timescale of this behaviour can vary; some sequences can last for 20 seconds whereas others can last up to 3 minutes. To view an example of this behaviour sequence, watch our video (BHS video A, 2023)
	Behaviour 2:
1. 	This behaviour was observed repeatedly throughout the study period. Here, frogs were seen to move their hind limbs side to side while shifting their abdomen side to side at the same time; at the beginning of the behaviour, the movement is slow and then continuously accelerates to the end. Frogs were observed carrying out this behaviour either alone or close to a conspecific. The timescale of this behaviour lasts for approximately 10 seconds. The behaviour is shown from lateral (image 1) and frontal (image 2) aspects; red lines indicate the moving parts. To view an example of this behaviour sequence, watch our video (BHS video B, 2023 ; BHS video C, 2023)
2. 	

PCA identified a consistent behavioural grouping of movement, resting and refuge, with the latter being opposed to the former two. This combination of behaviours follows a typical bold-shy axis (e.g. Martin & Réale, 2008), with behaviours associated with sitting out (movement and resting in open areas) negatively correlating with hiding. Disturbance significantly and substantially affected multiple measured behaviours. The greatest number of significantly affected behaviours were seen in the most disturbed frog dens (dens 2 and 4; disturbance intensities C and D), and the fewest changes in the least disturbed groups (dens 1 and 3; disturbance intensity B; Fig. 6). Most strikingly was the enormous and significant reduction in hunting behaviour under all disturbance intensities, and reduction in social interactions (disturbance intensities C and D) and vocalisations (disturbance intensity C) under the highest

disturbance intensities. Frogs in the den with the highest disturbance intensity (den 2, intensity C) also showed a significant shift of behaviour away from bold-orientated behaviours (movement and resting) towards shy-orientated behaviours (refuge). These results correspond to anecdotal reports of mountain chickens from keepers suggesting that similar disturbance causes feeding behaviour to decrease and potential association between interference with frogs and reproductive failure (Jameson et al., 2019). However, our results show that the impact of disturbance goes beyond feeding and impacts other aspects of behaviour, especially where disturbance was direct rather than indirect, and at higher levels of disturbance intensity.

As behaviours were counted at an enclosure level, differences between disturbance intensities C (3/4 of frogs captured) and D (1/4 frogs captured) are likely the

result of the differential dilution of behavioural changes based on the number of anaesthetised frogs per group.

These results demonstrate that disturbance may have key impacts on behaviours involved in feeding and reproduction. Given that consistent captive reproduction, unnaturally small adult size and nutritional disease are frequently a challenge for the ex-situ conservation of this species (King et al., 2011; Tapley et al., 2015; Jameson et al., 2019), our data strongly suggest that disturbance for husbandry and especially health checks may be a contributing cause. In alignment with this, Bell (2002) found that a factor responsible for unsuccessful captive breeding in Archey's Frogs *Leiopelma archeyi* was regular handling.

mSPI was not significantly affected by disturbance in frogs exposed to indirect disturbance (dens 1 and 3; disturbance intensity B) compared with the baseline, or in one of the dens with higher disturbance intensity (den 2; intensity C) but was significantly reduced in the other directly disturbed group (den 4, intensity D; Fig. 6). Given that a lower mSPI is typically associated with better welfare (see above), this result is unexpected. It is possible that the increase in resting behaviour and reduction in refuge behaviour in den 4 caused frogs to spend more time out of the refuges and in other resource zones. It is unclear why direct disturbance did not have this effect (nor even an effect approaching significance) and interpretation of this result is therefore tentative. However, this result suggests that behavioural data combined with mSPI can provide a better understanding of the impact of husbandry than mSPI alone, and that reducing mSPI does not necessarily indicate positive change if it is caused by negative behavioural shifts.

Health checks are imperative, particularly as mountain chickens are known to develop potentially fatal diseases within captivity (Ashpole et al., 2021), but our results show a clear tension between the need for health checks and the need for minimising disturbance. Importantly, the effects of disturbance were not limited to those dens including frogs that were captured, but were identified for some behaviours in dens that were indirectly disturbed by keepers catching frogs in neighbouring dens. This result highlights the sensitivity of mountain chicken frogs to disturbance and suggests that captive colonies might be best maintained where disturbance can be limited. Keeper anecdotes suggest that feeding may be suppressed for up to one month in disturbed mountain chickens (Jameson et al., 2019; Michaels & Servini, pers. obs.). Our data are not able to assess latency to return to normal behaviour, but taking these anecdotes as a guide, behavioural impacts may be both substantial and long lived, particularly as some collections may disturb frogs on a more frequent than monthly basis (Michaels, pers. obs.), which may prevent return to normal behaviour from ever happening. Further research is needed to assess rates of recovery from handling to determine impacts of behaviour, and the time taken for frogs to recover to their normal activity patterns. The use of in-nest box cameras to elucidate behaviour exhibited within these chambers is

also recommended to better understand not only what the frogs do inside the boxes, but also to identify further effects of disturbance and other husbandry practices on behaviour.

In other anurans, disturbance from zoo visitors, handling and veterinary procedures are known to cause stress responses that may be detected through corticosteroid stress hormones and sex hormones (Narayan, 2013; Narayan et al., 2012a; 2013), behaviour (Boulton et al., 2021) and dermal bacterial communities (Antwis et al., 2014) and that these effects may be dependent on the degree of disturbance (Narayan et al., 2011; 2012b). However, inter-specific differences appear, based on very limited data, to exist in that some taxa do not show stress hormone responses to relatively invasive procedures (e.g. Antwis et al., 2014b). Impacts of stressors on behavioural repertoires and resource use have not, to the authors' knowledge, been investigated in other anurans. However, our data suggest that mountain chicken frogs mount a stress response to environmental disturbance, restraint and veterinary procedures, although stress hormone analysis, which was outside the scope of this study, would be useful to link behavioural to physiological changes. The impact of disturbance on reproductive behaviours seen in mountain chicken frogs in the present study may be mediated by the strong and fast-acting impacts of restraint on sex hormones in other anurans (Narayan et al., 2012).

Our methods are limited by the necessity to collect data at the enclosure level. Although we controlled frog numbers and sex ratios, the ability to follow individual animals would provide higher resolution data on behaviour and response to putative stressors compared with our data, as well as increasing sample size and enabling more detailed statistical analysis. We did see imperfect alignment in results between the two enclosures exposed to indirect disturbance (disturbance intensity B), which indicates some variation in response between dens. Individual-level data, or collection of data from more groups, would also allow better assessment of behavioural effects for this reason.

Overall, our data provide initial insights into nocturnal behaviours, activity patterns and budgets for mountain chicken frogs and demonstrate sensitivity to disturbance and restraint in this species. These findings may be incorporated into best practice captive husbandry protocols for the species. Despite the utility of our data, important blind spots include the behaviour exhibited by frogs while within nest boxes, and the behavioural recovery period after disturbance. Further research into these areas, as well as work to link behavioural outcomes to physiological and endocrine mediators, is strongly recommended.

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