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In Absentia—Can a Lack of Behaviour Be a Useful Welfare Indicator? An Application to the Captive Management of Livingstone's Fruit Bats, *Pteropus livingstonii*

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Abstract: Non-invasive behavioural indicators of welfare can be particularly useful for managing captive breeding populations of endangered species; these allow individual welfare to be monitored and reproductive success maximised without the need for capture and restraint methods. However, most studies focus on the behaviours whose presence or frequency can predict welfare issues; the absence of a behaviour is less frequently considered an indicator of welfare. Here, we investigate potential behavioural correlates with welfare-related health states in captive Livingstone's fruit bats (*Pteropus livingstonii*), a critically endangered species that can become obese due to restricted space and reduced activity rates compared with wild populations. In this study, behavioural data were collected on males (which are particularly prone to obesity). Hurdle models were used to separately determine the factors predicting the presence or absence of behaviour and the frequency of observed behaviours. Whilst significantly lower levels of vigilance were observed in males with a larger body mass, those with diagnosed health issues were significantly more likely to show an absence of locomotion and foraging behaviour. Males with a lower body mass were also more likely to show an absence of foraging behaviour. Our study demonstrates how the absence of a behaviour can be informative as to an individual's welfare state. This study has identified behavioural profiles that can be used to flag at-risk individuals, reducing the need for potentially stressful handling and improving our ability to safeguard the welfare of individuals within a large captive group.

Keywords: individual welfare; behavioural indicator; *Chiroptera*; hurdle models; obesity

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1. Introduction

Non-invasive welfare assessment tools are becoming increasingly popular as part of the routine management of captive animals. More traditional methods of welfare assessment involving handling or capture can result in short-term modifications in behaviour and physiology; these can result in long-term implications for individual welfare and physical health [1]. Behavioural indicators can be used as reliable non-invasive measures that provide detailed information on animals' welfare states, especially for poorly studied or endangered species that are routinely managed in captive environments [2,3]. While the assessment of an animal's welfare should be approached holistically by making use of a variety of welfare parameters [1,4], the application of behavioural indicators is often an integral component.

The Livingstone's fruit bat (*Pteropus livingstonii*) has been listed as critically endangered by the IUCN since 1996 [5]. Environmental changes, habitat destruction, and stochastic processes such as violent cyclones have reduced its wild population to around 1200 individuals with an estimated 113.6 km^2 range and 21 roosts in the Comoros Islands [6–10]. Little is known about the wild behavioural ecology of this species [8], meaning in situ conservation efforts are challenging. Extremely low population numbers prompted the creation of a captive breeding programme in 1992 by the Durrell Wildlife Conservation Trust and the Comorian government as part of a conservation strategy to ensure the maintenance of the populations' genetic diversity [11,12]. The population maintained since then at Jersey Zoo, Channel Islands, has remained the largest captive population worldwide for this species and is therefore an extremely important breeding population.

Ensuring high levels of individual welfare in relatively large captive fruit bat colonies such as this is challenging. Bats are highly active in the wild, with many species travelling hundreds of kilometres between foraging and roosting sites. Accelerometer data gathered on two wild individuals found that *P. livingstonii* travels on average between 9.5 km and 12.7 km daily [13]. In captivity, allowing individuals the opportunity to be active and maintain adequate fitness levels is essential for their health and potential for reintroduction [11,14,15]; body mass is strongly influenced by enclosure size, and hence opportunities for flight, in this species [16]. However, due to the complexity of *Pteropus* flight patterns, as described by Bell et al. (2019), flight can be highly limited by objects in their enclosure; increasing opportunities for flight in captive fruit bats is therefore not straightforward. Additionally, while wild individuals feed primarily on fruit, a captive fruit bat's diet is supplemented with other protein-rich foods such as eggs and primate pellets, which may result in weight gain and a reduced requirement for activity; *P. livingstonii* weighs on average 56% (327 g) more than those captured in the wild [17]. This can reduce individuals' reproductive fitness, potentially reducing the effective population size and impacting genetic diversity [11,14,18]. It is therefore important to improve our ability to identify poor health or welfare in individual fruit bats, allowing for the monitoring and support of specific individuals.

Certain health conditions, some of which appear to be linked with obesity, are known to be prevalent in the captive population of Livingstone's fruit bats. Heart disease affected 3.5% of the total Livingstone's fruit bat population held in zoos between 1992 and 2017, causing 14.8% (n = 13) of the deaths recorded within that period [17]. Of these 13 deaths, all of which were recorded after 2011, 12 of them were characterised as dilated cardiomyopathy in adult and geriatric males [17]. While there is no available information on the lifespan of wild *P. livingstonii*, captive individuals can live upwards of 20 years, indicating that reducing mortality caused by cardiomyopathy could significantly improve the success of this captive population. Cardiomyopathy is a non-reversible condition; preventative measures, such as increasing activity levels and adapting diet, are key to reducing resultant mortality [19]. However, identifying individuals at risk of or suffering from cardiomyopathy in *P. livingstonii* is not straightforward. Males appear to be more prone to cardiomyopathy since older dominant males are more sedentary and defend feeding territories [11], rendering them more at risk of obesity. It is further suggested that older, heavier individuals will struggle to fly in a restricted space, escalating the impact of obesity through reduced activity in a negative feedback loop [11,16]. While certain clinical signs, such as increased respiration rate, effort, and reduced activity, are attributable to cardiomyopathy, these often arise once the disease has progressed too far to effectively treat it [19].

Non-invasive indicators of the risk of cardiomyopathy would significantly improve our ability to safeguard individual health and welfare in captive *P. livingstonii*. Diagnosing cardiomyopathy clinically requires routine echocardiography; this involves regular capture, transport, and anaesthesia [18], all potentially stressful procedures. Cardiac function is also altered when individuals change from their recumbent to their roosting posture, affecting the results of the echocardiography [18]. At Jersey Zoo, four individuals were diagnosed with cardiomyopathy during routine health checks or cardiac screening checks before the onset of clinical signs, one of which (in 2017) was completely asymptomatic. Once diagnosed, survival times from the start of treatment until natural death from cardiac failure or euthanasia ranged from 2 days to 390 days, highlighting the importance of early diagnosis to alleviate clinical symptoms of the disease through veterinary treatment [19]. The identification of a behavioural profile associated with higher body mass (and with other health issues, such as changes to bone and soft tissue caused by fractures, wounds, and inflammation, that can restrict movement and would otherwise only be diagnosed by capture and handling) in captive male Livingstone's fruit bats will allow for the visual, non-invasive monitoring of individuals so that interventions can be initiated prior to the onset of cardiomyopathy and the escalation of other health issues.

This project aimed to identify behavioural measures, both in terms of their relative frequencies and absences, that are associated with higher body mass and other health issues in captive male Livingstone's fruit bats housed at Jersey Zoo. Behavioural observations were carried out over two periods of data collection. The relationships between behavioural durations, body mass (measured at least once per year during routine veterinary checks), and incidences of health issues (as diagnosed by veterinary staff during routine checks) within the population were then examined. It was predicted that heavier individuals would spend more time roosting and foraging and less time in locomotion within the enclosure. This is due to heavier males being more likely to be older, displaying their dominance by controlling territory and food resources, reducing the time and competition effort needed to access food, and therefore increasing the amount of time available for roosting and feeding. It was also predicted that individuals affected by health issues would be likely to show an absence of locomotion during observation times due to reduced mobility and an attempt to alleviate symptoms.

2. Materials and Methods

2.1. Study Population

The data for this project were collected by MJE at Jersey Zoo, Channel Islands, during two separate data collection periods. The 'Summer 2019' period consisted of 35 days of data collection between June and September 2019, and the 'Spring 2020' period consisted of 20 days of data collection between February and March 2020. The 'Spring 2020' data collection period was curtailed due to the restrictions imposed by the COVID-19 pandemic. Whilst behavioural data were collected for both males and females in each period (see [20]), this project focused on male behaviour. Data were collected for 20 male *P. livingstonii* individuals during the 'Summer 2019' period and 22 males during the 'Spring 2020' period (which constituted all the adult males housed with the general population at the time). Nineteen males were sampled during both study periods; two were only sampled during the 'Spring 2020' period, and one was only sampled during the 'Summer 2019' period. The age range of the sampled individuals ranged between 1 and 18 years. Individuals were visually identified by inter-individual differences such as ear notches, back patch shape, colouration, and prominent wing holes, which remained unchanged over time. Additionally, a radio-frequency identification device (RFID) could be used to scan each individual's unique passive identification transponder (PIT) tag to verify an individual's identity without physical contact with the bats. Individuals included in the data collection were at least eight months of age and nutritionally independent from their dams.

The *P. livingstonii* population at Jersey Zoo was housed in the 'Island Bat Roost', a heated enclosure composed of two joined agricultural polytunnels. The enclosure was 38 m long × 16 m wide × 4 m high, with a 1.5 m deep circular trench used to raise the maximum height to 5.5 m [20]. The main enclosure was covered mainly with *Ficus* sp. and *Tradescantia* sp. along the ground and sides [11]. The ceiling and walls comprised medium-density mesh and rope, while the keepers' walkway was covered in artificial turf. The temperature within the enclosure was maintained between 16 °C and 32 °C using industrial fans, a mister system, a 45 kW biomass hot-air heater, and considerable insulation [20]. Humidity levels in the enclosure varied between 65% and 95% throughout the data collection periods.

The study population was fed twice per day [11], at approximately 11:00 and 16:00, via dispensers suspended from the ceiling around the perimeter of the enclosure, as well as in short plastic gutters attached to the western wall [11,20]. Feeding during the 'Summer 2019' period consisted of a Mazuri leaf-eater primate diet (Mazuri Exotic Animal Nutrition, St. Louis, MO, USA) soaked in water and offered twice a week, with all other feeds composed of a mix of chopped up fruit and vegetables [11,20]. During the 'Spring 2020' period, every morning feed consisted of the Mazuri leaf-eater primate diet, while all afternoon feeds comprised chopped fruits and vegetables. On Sundays, the fruit and vegetable mixture was supplemented by hard-boiled eggs [20]. Nursing individuals, as well as those recovering from a medical procedure, were additionally fed a portion of banana each morning.

Body mass data were not available for four males within the population; these individuals were excluded from further analyses. All bats were weighed a minimum of once per year during a routine veterinary check-up, but some individuals were weighed more frequently. The last recorded weight available for each individual at the time of data collection was included in the analysis. For the majority of the bats (16), these weights were recorded in April and May 2019, i.e., within two months of behavioural data collection commencing. Whilst five bats' weights were last recorded between January and March 2019, the other two bats' weights were recorded in October and November 2018. Bats' weights are recorded opportunistically (e.g., when vet checks are due) to minimise the stress associated with capture, meaning there is variation in when these are recorded, but since all but two were weighed within six months of data collection, we deemed these weights to be sufficiently accurate for our purposes. Each bat was visually assessed three times per day by keepers so that any abnormalities could be identified and acted upon as soon as possible. Health issues of interest (abnormalities such as wounds, fractures, or inflammation) were recorded during annual veterinary checks; many of these were not visible to keepers by observation alone.

2.2. Data Collection

Behavioural data were collected by MJE from inside the enclosure, as described in full by [20], for five to six hours per day, between 9:00 and 17:00 on five randomly allocated days in the week, resulting in a total of 304 and 187 ten-minute focal observations [21] for 'Summer 2019' and 'Spring 2020', respectively. MJE spent ten days prior to commencing data collection learning to accurately identify individuals and behaviours within the study population, simultaneously allowing the bats to habituate to their presence within the enclosure. As keepers enter the enclosure for routine management on a regular basis, it is not believed that MJE's presence caused any additional stress to the population.

Prior to commencing data collection, the enclosure was split into 42 hypothetical zones, each measuring approximately 4.67 m wide × 2.7 m long, based on pre-existing, evenly spaced features within the enclosure (Figure 1). A random zone was selected to start data collection within, with focal sampling used to record one individual's behaviour for 10 min. Each individual in the zone was sampled in turn, from north to south within the zone, before progressing clockwise to the next zone. Individuals that had already been sampled were not sampled again within the same 24 h period. Each day, behavioural observations began from the neighbouring zone clockwise to the one where sampling ended the previous day. This method ensured that all individuals were sampled at different times of the day. In the case that the focal individual changed zones during data collection, the observer followed the individual, and data collection continued; they then returned to the original zone to observe the next individual in that zone. If locomotion behaviour caused the observer to lose track of the individual, behavioural observations were halted, and the individual was recorded as out of sight for the remainder of the focal sample.

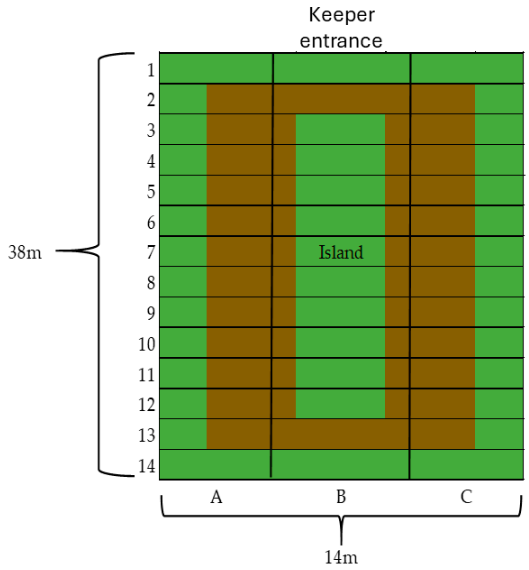

Figure 1. This figure depicts an aerial representation of the "Island Bat Roost" at Jersey Zoo, Channel Islands. The brown sections show the 1.5 m trench surrounding a central island, which included a barrier around which bats had to fly. The 42 artificial sections used for this study are denoted by 14 rows (1–14) and 3 columns (A–C).

The durations of behaviours performed by the focal individual were continuously recorded in seconds during the ten-minute sampling session using the Animal Observer (version 1.0) iPad application. The recorded behaviours were defined according to an ethogram based on descriptions developed by [22] Courts (1996) and Welch (2020) for *P. livingstonii* in captivity (Table 1).

Table 1. Ethogram of studied behaviours and sub-categories for captive *P. livingstonii*, modified from Courts (1996) and Welch (2020). There was also an "Other" category for all behaviours not included here. This ethogram does not include every behaviour recorded during data collection, only those included in our analysis.

Behavioural Category	Sub-Category	Description
Vigilant	Bipedal hang	Hanging by both hindlimbs, with wings either wrapped around the ventral region or folded on either side of the body. Eyes are open, and there may be ear movement, air sniffing, and head movement.
	Quadrupedal hang	As bipedal, but hanging by all four limbs, wings folded on either side of the body.
Foraging	Drink	Using the tongue to ingest water from a water dish or to lick moisture off the walls.
	Feed	Ingestion of food items. Larger pieces of hard food items may be held by a hindlimb whilst licking or biting at food.
	Investigate	Sniffing food items with the nose or sniffing within 30 cm of them.
Locomotion	Climb	Movement from one area of the substrate not directly connected to another, accomplished by the use of both forelimbs to hook onto the substrate.
	Flight	The flapping of both wings to achieve horizontal movement through the air.
	Mesh movement	Forward movement on a horizontal mesh, using fore and hindlimbs. The forelimb is followed by the opposing hindlimb, followed by the opposite forelimb, etc.
	Floor movement	A slow crawl, using opposite fore and hindlimbs in succession, along the ground.
Roosting	Roost alone	Hanging by a single hindlimb, wings wrapped around the ventral region, and muzzle tucked into the chest. Eyes are closed; no ear movement.
	Roost in pairs	As roost alone, but with one bat wrapped in the other's wings.

2.3. Data Analysis

All statistical tests were carried out in the R environment [23]. Data for the subcategories of behaviour were combined into their parent behavioural category (i.e., Vigilance, Foraging, Locomotion, or Roosting). The proportion of time during which the focal individual carried out a behaviour within each category was then calculated for each 10 min sample; a proportion was used since some samples were not full 10 min samples due to an animal moving out of sight or moving too far away for the observer to follow their movements; hence, using proportions made samples comparable. On examination of these data, zero-inflation was apparent as frequently the focal individual did not carry out a particular behaviour at all (81.5% of samples did not include foraging behaviour, 48.8% did not include locomotion, 39.7% did not include vigilance, and 31.6% did not include roosting). We therefore chose to employ hurdle models [24], so that the factors contributing to the positive proportion of time a behaviour was carried out could be investigated separately from those increasing the likelihood that the behaviour would not be carried out at all (i.e., was given a zero value).

The hurdle model we used included a GLMM to account for repeated measures (in this case, the focal ID) applied to the positive values and a binomial model to account for the probability of a zero value. We used the "mixed_model" function in the GLMMadaptive package [25] to fit a GLMM (truncated at zero) with the lognormal distribution as its family (the best fit to our dataset). The fixed factors in the full model were the data collection period ('Summer 2019' or 'Spring 2020'), health issues (a binary measure of whether an individual had been diagnosed with a soft tissue or bone abnormality), and body mass. Age was found to covary with body mass and was therefore excluded as a factor. Whilst males with a larger body mass were also significantly more likely to have a diagnosed health issue ($W = 9.971$, $df = 1$, $p < 0.05$), three individuals had health issues alongside a lower-than-average body mass; since our aim was also to identify individuals with a health issue independently of those with a large body mass, we included both health issue and body mass as separate factors. Four separate models were built, where the response variable was the proportion of time when the individual was carrying out vigilance, foraging, locomotion, or roosting behaviour, respectively. The focal individual's identity (ID) was included as a random factor in each model as there were multiple observations of the same individuals.

Five other biologically relevant models were also tested with different combinations of the fixed factors (see Supplementary Materials). The Akaike Information Criterion [26] was calculated for each model. The model with the lowest AIC was selected as the best model, together with any within two ΔAICs of this model, as both would have strong statistical support; where two were equally likely, the simplest model was selected as the best model [27].

3. Results

After removing data for four males whose body mass was unknown, a total of 453 focal samples remained (304 in 'Summer 2019', 149 in 'Spring 2020'). The median body mass of the 20 males whose data were analysed was 820 g (min 650 g, max 1100 g), and 11 of these males had been diagnosed with health issues by veterinary staff prior to data collection commencing. Across all samples, individuals spent on average 38.4 ± 38.7% of their time roosting, 17.1 ± 18.7% of their time vigilant, 13.5 ± 16.8% of their time in locomotion, and 6.9 ± 18.4% of their time foraging (values are mean ± sd). The remainder of the bats' time budgets were spent on other behaviours.

The best model for each behaviour was selected by using the Akaike Information Criterion (see Supplementary Materials for models tested and their corresponding AIC values; for full results from the best models, see Table 2).

Where vigilance behaviour occurred, body mass significantly negatively predicted the proportion of time spent vigilant, although the effect size was small ($\beta = -0.008 \pm 0.000$, $p = 0.008$; Figure 2). An absence of locomotion behaviour was significantly more likely in individuals with a diagnosed health issue ($\beta = 0.267 \pm 0.097$, $p = 0.006$; Figure 3);

there was also a data collection period effect on this behaviour as an absence of locomotive behaviour was more likely during the 'Spring 2020' period (Table 2). An absence of foraging behaviour was also significantly more likely in individuals with a diagnosed health issue (β = 0.739 ± 0.252, p = 0.004; Figure 4); the absence of foraging was also significantly more likely in those with lower body mass, with a smaller effect size (β = −0.004 ± 0.001, p = 0.005; Figure 5). There was a data collection period influence on roosting behaviour; individuals spent a greater proportion of time roosting during the 'Spring 2020' period (Table 2).

Table 2. Coefficients for best models used to determine behavioural predictors of weight and/or health issues in Livingstone's fruit bats using the hurdle model approach. Estimates for each coefficient ± its corresponding standard error are presented for positive values (duration), i.e., the part of the model that explains the relative time spent carrying out a behaviour given the fact that it is occurring, and zero values (binary), i.e., the binary part of the model that shows which factors predict the binary presence or absence of a behaviour, separately. Factors with significant effects ($p < 0.05$) are indicated by bold text. 'Summer 2019' was the baseline condition for the period (one of two data collection periods). Where a factor was not included in the best model for a particular behaviour, we have inserted "-" to indicate there was no coefficient available for this factor.

Behaviour	Intercept	Body Mass	Health Issue	Period
Vigilance (duration)	−0.755 ± 0.251	**−0.008 ± 0.000**	0.064 ± 0.037	−0.066 ± 0.074
Vigilance (binary)	0.114 ± 0.759	0.000 ± 0.001	−0.092 ± 0.112	−0.499 ± 0.204
Locomotion (duration)	−1.459 ± 0.089	-	−0.068 ± 0.051	0.006 ± 0.088
Locomotion (binary)	0.162 ± 0.182	-	**0.267 ± 0.097**	**−0.644 ± 0.205**
Roosting (duration)	−0.341 ± 0.332	0.000 ± 0.000	-	**−0.282 ± 0.101**
Roosting (binary)	−2.440 ± 0.767	0.002 ± 0.001	-	**0.448 ± 0.225**
Foraging (duration)	−1.643 ± 0.930	0.000 ± 0.000	0.126 ± 0.189	-
Foraging (binary)	4.321 ± 1.112	**−0.004 ± 0.001**	**0.739 ± 0.252**	-

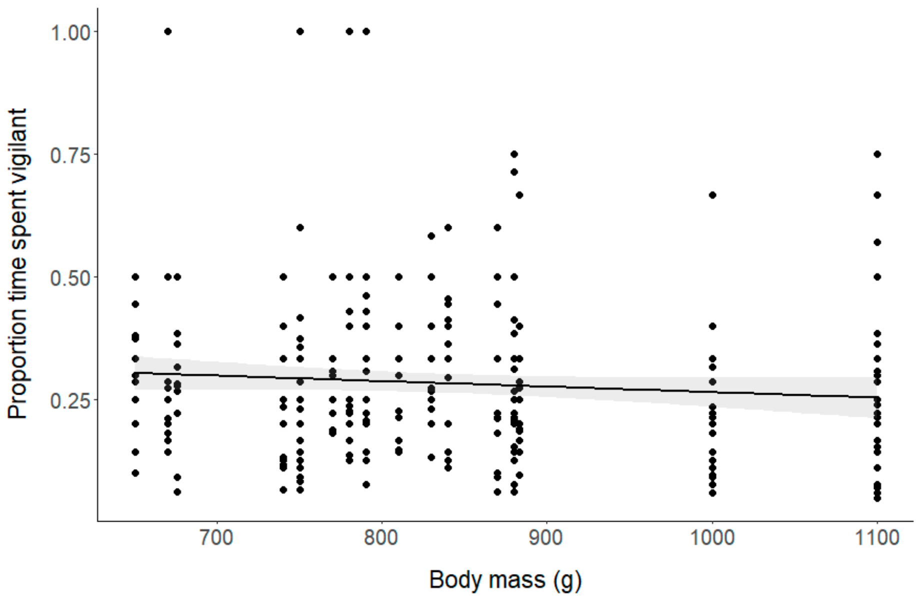

Figure 2. Where vigilance behaviour was observed, Livingstone's fruit bats with a larger mass spent significantly less time engaged in this behaviour. The y axis shows the proportion of time spent vigilant in each focal sample. Each individual point represents one focal sample. Since some bats had an identical body mass, there are larger numbers of points illustrated for these masses to reflect the individual sampling points. The black line represents the direction of the relationship defined by the model (see Table 2). Shaded areas represent 95% confidence intervals around the estimates.

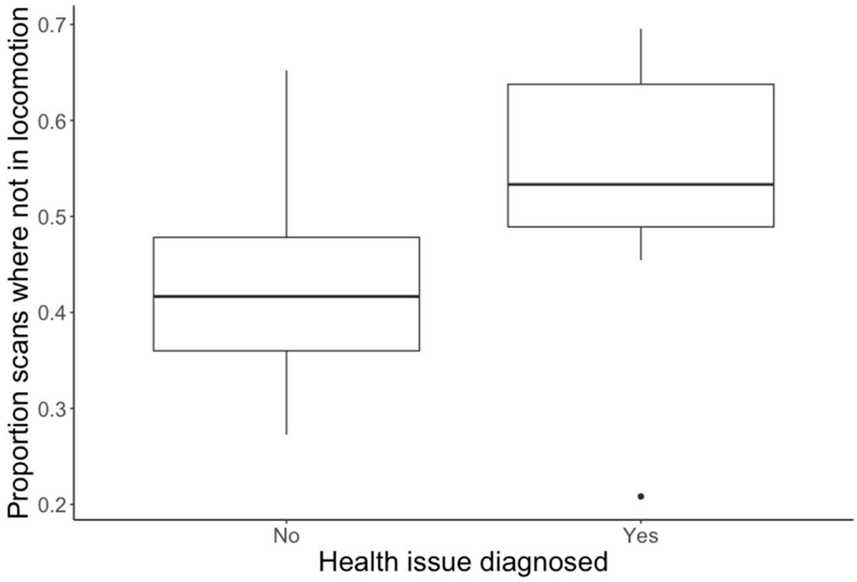

Figure 3. An absence of locomotive behaviour was significantly more likely to be recorded for individuals with a diagnosed health issue during behavioural observations of Livingstone's fruit bats. Eleven individuals had diagnosed health issues out of a total of twenty-two individuals. The boxes' boundaries indicate the interquartile range. The boxes are bisected by a line indicating the median, and their whiskers indicate 1.5 times the interquartile range. Outliers are indicated by points.

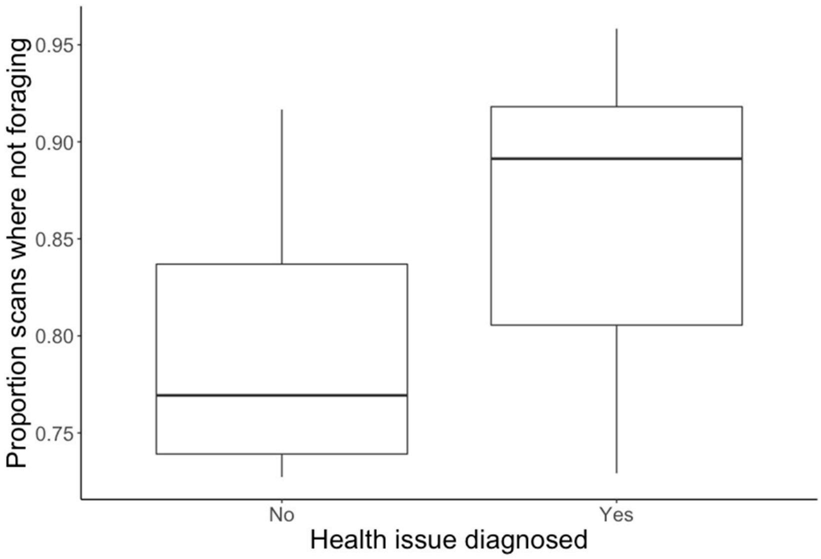

Figure 4. An absence of foraging behaviour was significantly more likely to be recorded for individuals with a diagnosed health issue during behavioural observations of Livingstone's fruit bats. Eleven individuals had diagnosed health issues out of a total of twenty-two individuals. The boxes' boundaries indicate the interquartile range. The boxes are bisected by a line indicating the median, and their whiskers indicate 1.5 times the interquartile range.

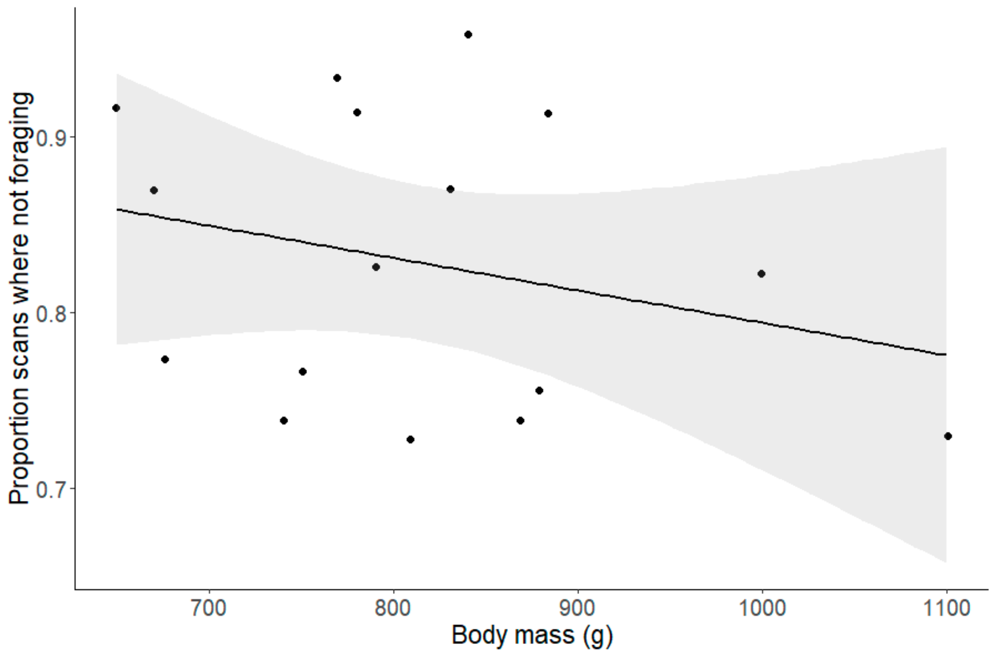

Figure 5. An absence of foraging behaviour was significantly more likely to be recorded for individuals with a lower body mass during behavioural observations of Livingstone's fruit bats. The black line indicates the direction of the relationship defined by the model (see Table 2). Shaded areas represent 95% confidence intervals around the estimates. Each data point represents one individual, and the y axis shows the mean proportion of all scans where that individual was not recorded as foraging.

4. Discussion

This project aimed to identify behavioural correlates with both larger body mass (related to a higher risk of cardiomyopathy) and other health issues, such as bone and soft tissue abnormalities caused by wounds, fractures, or inflammation, in captive male Livingstone's fruit bats, providing keepers with a new tool to non-invasively monitor welfare in this critically endangered species. Hurdle models were employed to identify two types of indicators: on the one hand, behaviours whose relative prevalence could indicate health issues or potential obesity, and on the other, behaviours whose absence could predict the likelihood that these conditions were present. Both could be useful measures for animal carers to non-invasively monitor individual welfare in this and other captive populations.

An absence of foraging behaviour was found to be associated with both a lower body mass (although the effect size for this relationship was small, Figure 5) and the presence of a health issue (Figure 4). Since age correlates with body mass in this species, older individuals are more likely to be dominant within the population [28] and control more foraging territory [15], leading to an increase in time spent foraging around those areas, whilst younger subordinates that do not hold a territory take higher risks of experiencing aggression when foraging and consequently do so less frequently [28]. This could explain the relationship between lower body mass and the number of periods when no foraging was observed. Interventions could be initiated to provide individuals with a lower body mass more opportunities to feed; for example, O'Connor (2000) [29] found that providing mealworm dispensers as an enrichment tool increased the amount of time individual Rodrigues fruit bats at Jersey Zoo spent foraging and feeding, while increasing general activity levels around the feeders and decreasing the prevalence of agonistic interactions. Providing more unpredictable food sources could also require dominant individuals to feed more infrequently, with potential health benefits in terms of reducing the likelihood of obesity. However, since individuals with a health issue were also more likely to show an absence of foraging behaviour, keepers could use this behaviour's absence, particularly in more dominant individuals, as an indicator of potentially poor welfare. If individuals that are not seen to forage are flagged, they could be monitored more closely to ascertain the reason for the lack of this behaviour, especially if they are a relatively high-ranking individual.

In this study, we identified the absence of locomotion behaviour as a reliable indicator of the presence of health issues (Figure 3). This could be a direct result of individuals' poorer abilities to move around the enclosure due to conditions affecting mobility in their limbs and digits; it could also indicate an individual is experiencing pain or that the metabolic demands of locomotion are not able to be met due to illness or injury. We expected decreased levels of locomotion to correlate with higher body mass. Bell et al. (2019) found that individuals of this species that were less than three years old were observed flying most frequently, followed by individuals between three and ten years old (which would have a larger body mass). Bats older than ten years old were not observed performing any flying behaviour, possibly due to their dominance rank, which renders them more likely to be sedentary and control a territory [17,27]. It has recently been shown that bats living in smaller enclosures are more likely to have a higher body mass, presumably due to their limited opportunities to fly [16], again supporting our expectation of reduced locomotion correlating with higher body mass. Additionally, reluctance to fly and lethargy are two of the clinical signs of cardiomyopathy in *Pteropus* species described by Killick et al. (2017). The likely explanation for a lack of association between body mass and locomotion behaviour in our analysis is that locomotion was not split into flying and other locomotory behaviours; it could be that individuals that are less able to fly show higher levels of climbing to compensate for this deficiency. An absence of locomotion could therefore be a useful indicator that an individual might be suffering from a health issue, such as an injury, flagging to keepers that further observation or a health check is required, but is not necessarily linked to the risk of obesity in this species.

Where vigilance behaviour was observed, individuals with a higher body mass showed decreased levels of this behaviour, although the effect size was small (Figure 2). Since vigilance is likely to be towards conspecifics, younger individuals with a lower body mass that are likely to have a lower dominance rank might need to spend more time monitoring other individuals' movements to avoid costly aggressive confrontations, as dominant individuals are likely to instigate agonistic interactions towards subordinates to maintain their rank [30]. This would be especially true when subordinates are foraging as they might need to enter a dominant individual's territory; bats that frequently move between social groups are known to have higher cortisol levels in this species, highlighting that perceived levels of risk can be higher in these individuals [30]. Individuals with a larger body mass are likely to be showing reduced vigilance behaviour as they are more likely to be territory holders and therefore hold a higher dominance rank (as body mass, age, and dominance rank are positively correlated in this species; [16,28], with less need to socially monitor others. Particularly low levels of vigilance behaviour could indicate higher body mass in this species, meaning this behaviour could be used by keepers as an indicator of risk of cardiomyopathy; males that are showing low levels of vigilance have more time for foraging and therefore weight gain.

This study has highlighted that there can be independent explanations for the relative frequencies of expressed behaviours and the probability that these behaviours are expressed at all. Whilst the former is commonly investigated in behavioural studies, using hurdle models to separately identify the potential explanations for an absence of behaviour could be an extremely useful addition. Our results show that the lack of expression of a certain behaviour may be as useful a measure of welfare as the level of expression of another. They have also shown that whilst body mass correlates with the likelihood of developing certain health conditions in this species, different behaviours are associated with larger body mass compared with the incidence of other health issues; identifying individuals at risk from each of these separately can help provide more targeted interventions. Noticing these behavioural differences in individuals may allow early detection of cardiomyopathy, permitting the provision of treatments that alleviate the clinical symptoms of the disease and increasing the welfare of affected individuals [19]. This could also reduce the requirement for potentially stressful routine echocardiograms, since only at-risk individuals need to be subject to this procedure. However, recording the absence of an expected behaviour could require more time commitment from keeping staff; the development of activity monitoring technology for fruit bats in the future has the potential to facilitate this process via automated monitoring.

Whilst results from this study suggest that lower levels of vigilance behaviour in males correlate with a higher body mass and that an absence of locomotion or foraging could indicate other health issues, more data are required for firmer conclusions to be established. Since this colony represented around 80% of the captive population for this species at the time of data collection, there is little scope for increasing the number of individuals sampled; however, sampling across more data collection periods and over a longer timeframe could be useful. The current literature suggests Livingstone's fruit bats are crepuscular, with foraging behaviour peaks between 2200 h and 0200 h [31], suggesting that short daytime observations may not be fully representative of a bat's true activity patterns. Future research should aim to conduct continuous focal observations over 24 h to gain a better understanding of the species' behavioural patterns in different demographic groups. Recording such data could be particularly useful if changes in behaviour are recorded immediately preceding the onset of a particular health condition, so these can be used as a true early warning indicator.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jzbg5020016/s1, Table S1: Factors included in the biologically relevant models tested to test for relationships between body mass, data collection period, and the presence of a health condition on four discrete behaviours in Livingstone's fruit bats.

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