The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK
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Physiological and behavioural assessments of stress levels in owls housed at owl cafes

C Urita†, S Kusuda‡ and N Rooney*†

† Bristol Veterinary School, University of Bristol, Langford BS40 5DU, UK
‡ Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido Gifu-City, Gifu Prefecture, Japan
* Contact for correspondence: Nicola.Rooney@bristol.ac.uk

Abstract

*Owl cafes, where customers view and interact with owls, have become popular in Japan. There are multiple aspects of the environment which may be stressful to nocturnal owls, including lighting, tethering and frequent interactions with humans but, to date, welfare has not been investigated. This preliminary study examines the effects of owl cafes and customers on the physiological stress (faecal corticosterone levels [FCL]) and behaviour of the owls. Seven eagle owls (*Bubo bubo*) and two African scops owls (*Ptilopsis leucotis*), in two cafes, were studied over an eight-day period. Cafe A (n = 5) was closed for one day per week, whilst cafe B (n = 4) was open every day. In cafe A there was higher FCL in owls during open days than closed days suggesting that the conditions on open days increase stress in owls. Eight of nine owls showed evidence of some aversion or avoidance of humans, whilst no affiliative behaviours were observed. The number of visitor interactions was not associated with the level of physiological stress; and when comparing owls, there was a tendency for individual owls with a higher percentage of aversive responses to customers to also have higher FCL. Close human interactions of a negative nature, that result in aversion, may be a significant stressor, but further research is required.*

Keywords: *African scops owl, animal welfare, behaviour, corticosterone, eagle owl, interaction*

Introduction

In Japan, since 2010, cafes in which people are able to interact with animals, including exotic species such as owls, have gained in popularity and there are now over 50 establishments housing over 200 owls. These cafes generally have tethered owls, are open in the day and allow customers to interact and even pick up the birds. For nocturnal, non-domesticated animals, this is likely to be stressful. Hence, it may be questioned whether a customer-centric, daytime cafe constitutes a significant welfare concern for owls.

Potential welfare issues include captive conditions and extensive interactions with humans. Although, some wild species experience a healthier and longer life and breed more successfully in captivity, due to enhanced veterinary care and food and water provision (Mason 2010), keeping wild species in captivity often has negative effects especially when environmental conditions differ greatly from the wild. Examples of this would include wide-ranging carnivores that see a drastic restriction in their home ranges (Clubb & Mason 2003) and tropical animals and birds whose thermal and UV requirements are not replicated in captivity (Ross *et al* 2013). A potentially significant stressor in cafes is exposure to humans, from which the owls cannot escape. Almasi *et al* (2015) examined how wild barn owl (*Tyto alba*) nestlings were affected by anthropogenic activities around their nest sites, and saw that nestlings subjected to frequent human disturbance exhibited increased plasma corticosterone concentrations and achieved smaller body masses. In contrast, a study by Dufty and Belthoff (1997) suggested that captive western screech owls (*Otus kennicottii*) did not experience high levels of stress during human handling (eg capture) compared to free-living owls, although more recent, advanced measures of stress may find otherwise. Most of the owls kept in cafes are captive-born, although some are rescued from the wild, post-injury. Conditions in owl cafes include multiple stressors including large numbers of unfamiliar humans often touching the owls. In addition, most of the owls are tethered. It is generally advised that tethering be carried out in conjunction with flying and time spent in an aviary (Parry-Jones 2008), in order to promote optimal welfare, however this is seldom the case for owls in cafes. It is important therefore that the welfare of owls housed in cafes is investigated. Research has taken place on the implications of exotic animal cafes from the perspective of conservation (McMillan *et al* 2020), but there are yet to be any studies examining the welfare of owls in cafes.

We observed nine owls, housed in two cafes each over eight days. In birds, physiological stress can be measured using faecal corticosterone assays. It has been shown in various species, including owls, that faecal corticosterone levels (FCL) reliably reflect adrenal activities, which are activated by stressors (Wasser *et al* 2000). To evaluate the potential impact of stressors in the cafe environment, we compared the birds' behaviour and FCL when the cafes were open as compared to closed. We also compared the behaviour and stress of owls on days with different customer levels, and numbers of customer/owl interactions. Finally, we compared the behaviour of individual owls, both in the absence of customer(s) and when approached by customer(s). We predicted that those showing the greatest aversion to people, and those showing the highest levels of negative behaviours when undisturbed, would also have the highest FCL.

The hypotheses tested were:

• Owls will have higher FCL on open compared to closed days;

• The percentage of time spent in positive and negative behaviours when undisturbed (in absence of customers on open days) will correlate with the individual owl's FCL;

• An individual owl's FCL will positively correlate with the Percentage of Aversive Reactions it shows to customers (PAvR); and

• There will be a positive correlation between FCL and the number of customer and human interactions an owl experiences on a given day.

Materials and methods

Study site and target animals

The subjects were housed in two cafes (A and B) in the Tokyo Metropolitan Area and Kanagawa Prefecture of Japan. The cafes were similar sizes; 30 and 31 m^2, respectively. We studied the two species that were common to both cafes: four eagle owls (*Bubo bubo*) and one African scops owl (*Ptilopsis leucotis*) in cafe A and three eagle owls and one African scops owl in cafe B (Table 1). Cafe A had just the five owls, but also three hawks, whilst cafe B had 30 more individuals of 25 different species. These were not studied as they could not be compared between cafes and there were too few representatives of each species, to provide meaningful data. All owls except one (Table 1) were tethered separately and were given water by spraying, every 1–2 h in cafe B or when either showed behavioural signs of thirst (opening mouth) in cafe A. Both cafes were open for 7 h per day, but cafe A had one closed day per week, while cafe B was open every day. As well as there being customers present on open but not closed days, the lights were off on closed days (except when the staff fed the birds) and the locations where the owls were tethered differed on closed days, compared to open. In addition, Owls 3 and 4 were taken to the owner's home and tethered when the cafe was closed. When shut overnight, cafe A's owls remained tethered, whereas cafe B's owls were placed in individual cages each with a water bowl. Lights were off overnight, although there was some illumination from an emergency light and the street-lights in cafes A and B, respectively.

Faecal corticosterone measurements

Sample collection

Faecal samples were taken from all nine owls on eight open and two closed days in cafe A and eight open days in cafe B. Collection of faecal samples began 90 min before closing time on open days in cafe A and continued until the cafe closed. Sampling times were matched on the two closed days. According to the research conducted by Wasser and Hunt (2005), great horned owls (*Bubo virginianus*), whose genus is the same as eagle owls, excrete most hormones, including corticosterone, in faeces within several hours after their secretion in blood. Thus, the sampling timing was considered appropriate to measure stress, during the day's opening hours. Faeces were picked up as soon as possible after defaecation without interrupting customer/owl interactions, using aluminium foil and wood sticks. Samples were wrapped in aluminium foil and placed into a sealed bag. They were transported in an ice box, placed in the freezer (–20°C) within 2 h of collection, and stored until shipment via a frozen delivery service. Samples were subsequently stored in the freezer (–20°C) until processing. Extraction and assay of corticosterone was carried out at Gifu University, Japan.

Extraction and assay

The samples were placed in an oven (100°C) for approximately 30 min to be dried. We weighed out 0.1 g of the sample; if the weight was less than 0.1 g, we used as much as possible, to the nearest 0.02 g (ie 0.08, 0.06, 0.04 and 0.02 g). Due to small volumes (less than 0.02 g), 17 samples were unusable, leaving 65 samples. We added 5 ml of methanol (80%) per 0.1 g of sample. The samples were shaken for 30 min using a test-tube mixer, and centrifuged at 2,500 rpm for 10 min at 4°C. The supernatant was transferred into a clean tube and stored in a freezer (–20°C) until assayed.

The extracted supernatant was assayed by enzyme immunoassay using DetectX CORTICOSTERONE Enzyme Immunoassay Kit (Arbor Assays, Michigan, USA), according to the manufacturer's instructions. A 1:10 dilution was initially employed for samples, but a number of minimum values (39.06 ng g^{-1}) were detected, suggesting the dilution factor was too great. We therefore employed a 1:5 dilution and repeated the assay three times. The average FCL in each sample was calculated from these four assays including 1:10 dilutions. Any minimum values at 1:5 dilution were given a score of 19.53 ng g^{-1} and the minimum values of the 1:10 dilution were eliminated from the average calculations.

Customer interactions

A single observer (CU), positioned where all focal owls were visible, recorded all human interactions occurring with each focal owl during opening hours (7 h per day) for each of the eight days in each cafe. A customer interaction was defined as: customer(s) moving to within 1 m of an owl for more than 5 s with or without touching an owl. We separately recorded carrying events when a customer placed an

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Table 1 Individual owls listed in each of the two cafes; their species, sex, age, age on arrival at the cafe, and other relevant information.

Cafe	Owl ID	Species	Sex	Age (years)	Age on arrival at cafe	Tethered	Duration of customer absent observations (min)	Other info
A (with closed days)	1	American eagle owl	F	Unknown	Unknown (but adult)	Y	111	Rescued individual
	2	African eagle owl	M	2	Unknown (but < 1 year)	Y	111	Bought by customer, formerly a pet but returned to the cafe this year
	3	Bengal eagle owl	M	2	Unknown (but chick)	Y	111	Stays at owner's house outside opening hours
	4	Eurasian eagle owl	F	2	Unknown (but chick)	Y	108	Stays at owner's house outside opening hours
	5	African scops owl	M	2	Unknown (but chick)	Y	105	
B (with no closed days)	6	Cape eagle owl	M	2	4 months	N	66	Not tethered. Always stays at high position out of the reach of customers
	7	African eagle owl	M	2	3 months	Y	63	
	8	Siberian eagle owl	M	1	1 month	Y	60	
	9	African scops owl	M	4	2 years	Y	57	

Owls' sex reported by cafe staff.

Table 2 Classification and definitions of owl reactions to customer interaction.

Classification	Individual reaction	Definition
Affiliative (PAfR)	Approach	Getting or attempting to get closer to the customer(s) without aggression
Aversive (PAvR)	Aggression	Grabbing or tackling with feet, or biting (Park 2003)
	Alert	Staring and/or bobbing or back turning (Kumar 1985) or taking defensive postures (Park 2003)
	Avoid	Trying to stay away from the customer(s) or dodging the customer's touch
Neutral (PNR)	No reaction	No change in activity

owl on their shoulder or arm. An interaction began when the customer started to approach the owl and ended when the customer left. When two or more customers were present, the event ended when the last customer left. The number of customers in each event were counted. When customers interacted with two or more owls at the same time, the event was considered as one event for each owl.

Over each day, we calculated the total Number of Customers Present in the cafe (NCP), and for each owl the Number of different Customers that Interacted (NCI), the Number of Interaction Events (NIE), and the Number of Carrying Events (NCE). NCI could be larger than NCP, because customers could interact with the same owl multiple times.

The immediate reaction of the owls to each customer interaction were recorded (Table 2). The percentages of affiliative, aversive and neutral reactions by each owl were also calculated (Table 3).

Owl behavioural observations

Continuous focal sampling was also conducted to assess the time each owl spent in different behaviours when undisturbed (eg the cafe was closed or no customers present). Observations were conducted at sufficient distance, and after a habituation period and were not observed to disturb the owls. Each sampling block was 3 min in length and terminated when customers arrived. Twenty different behaviours were recorded (Table 3) using a phone timer and

Table 3 Classifications and definitions of owl behaviours observed during focal observation.

Classification	Individual behaviour	Definition
Resting	Sleeping	Closing eyes partially or completely (Kumar 1985)
Neutral behaviour	Looking at one point	Stares in one direction apparently watching something
	Walking	Movement with feet on the ground or a perch
	Flying	Movement through the air with the wings or attempt at the same
	Feeding	Eating the feed
Positive behaviour (comfort and maintenance)	Bill cleaning	Wiping the beak sideways on a perch with closed eyes (Kumar 1985)
	Preening	Grooming feathers with the bill (Kumar 1985)
	Scratching	Rubbing areas of the head or neck (Kumar 1985)
	Wing fluttering	Flapping wings (Kumar 1985)
	Body fluffing	Erecting feathers and then shaking the body (Kumar 1985)
	Toe/leg cleaning	Pecking and biting the toe or leg softly with the beak (Kumar 1985)
	Stretching	Extending the wing and/or leg fully (Mahmood-ul-Hassan 2008)
	Playing	Holding something (eg cardboard) with the beak or foot and biting it or shaking the head
Negative behaviour (known stress behaviours)	Back turning	Turning the head around by 180° to look at the intruder (Kumar 1985). One of the alert behaviours (Kumar 1985)
	Staring and/or bobbing	Moving the head up and down and/or with gazing with wide-opened eyes. One of the alert behaviours (Kumar 1985)
	Sleeking plumage	Drawing feathers in tightly, looking like a part of a tree (Ramanujam 2004)
	Gular fluttering	Opening the mouth and trembling the throat (Park 2003). Observed when the owl feels stressed or hot (Park 2003)
	Chewing tether	Biting the tether aggressively
	Agitated alert	Looking around restlessly with wide-opened eyes
	Wing drooping	Lowering both wings (Park 2003). Observed when the animal feels hot or stressed (Park 2003)

data-sheets. Self-maintenance behaviours such as preening and play behaviours were classified as positive behaviours, as they are known to decrease when animals are under stress (eg Duncan & Wood-Gush 1971; Olsson *et al* 2011). Agitated alertness can be a sign of excessive vigilance and was categorised as a negative behaviour along with the known stress behaviours of beak turning, staring and bobbing (Kumar 1985), sleeking plumage (Ramanujam 2004) and gular fluttering (Park 2003). The proportion of time spent resting and engaging in positive, negative and neutral behaviours was calculated for each owl.

Data analysis

IBM SPSS Statistics (Version 23) was used for statistical analysis. Summary statistics were calculated for corticosterone, focal sampling, number of customer interactions and owl reactions. Normality of the data was assessed via Kolmogorov-Smirnov tests, and histograms. FCL did not show normality, so were transformed using Ln(x+1). Independent samples t-tests were used to test for differences in FCL between cafes. They did not differ significantly, so were analysed together in the closed vs open comparison. Univariate General Linear Model (GLM) analysis was used to test the effect of closed vs open days on corticosterone for owls within cafe A, with owl ID and closed/open as fixed factors, and their interaction included. To compare FCL to customer levels across days, we ran four separate Univariate GLMs with owl ID and each of NPP, NPI, NIE and NCE as fixed factors, in turn. Individual owl's FCL was compared to their behaviour (in the absence of customers and during interactions) using Spearman correlation tests.

Ethical approval

The ethical approval for this study was granted by the University of Bristol Animal Welfare and Ethical Review Body (approval reference: UB/17/041).

Results

On an individual day, the number of customers, at each cafe ranged from 1 to 95. The total number of customers in cafe B was almost nine times that of cafe A with 492 over the eight days, compared to just 58 (Table 4).

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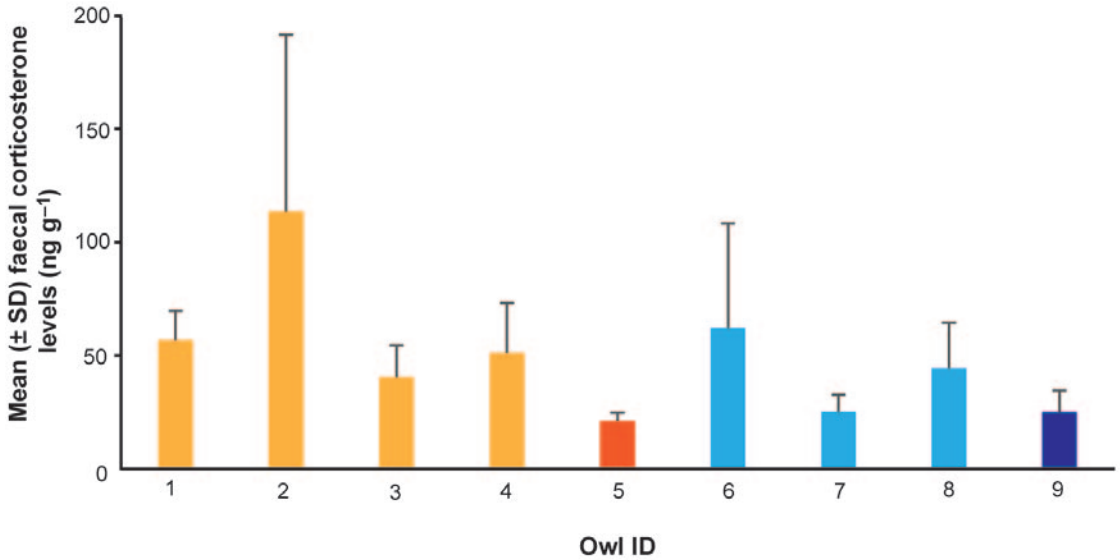
Table 4 Information of each cafe.

Cafe	Number of closed days per week	Opening hours	Total customer numbers over eight open days	Average customer number per day
A	1	1330–2030h	58	7.25
B	0	1200–1900h (weekdays) 1300–2000h (weekends)	492	61.5

Figure 1



Mean (± SD) faecal corticosterone levels for each owl. Orange bars (Owls 1–5) represent owls in cafe A, blue bars (Owls 6–9) show owls in cafe B. Lighter bars represent eagle owls and darker bars, African scops owls.

Corticosterone assay

We could not collect faecal samples from all owls, on all sampling days, because some did not defaecate within the collection time or the faecal samples were too small to analyse. Overall, 65 samples were used for analysis.

The mean (± SD) FCL was 51.45 (± 41.31) ng g^{-1} and ranged from 19.53 to 266.72 ng g^{-1} (Figure 1). The mean (± SD) FCL for cafe A was 57.39 (± 45.81) ng g^{-1} (n = 42), and 40.60 (± 29.36) ng g^{-1} (n = 23) for cafe B; the difference was not statistically significant ($t = 0.39$, df = 63; $P = 0.12$). The mean FCL in the two species differed significantly ($t = 5.55$, df = 61.26; $P < 0.05$); with eagle owls generally being higher 57.89 (± 43.13) ng g^{-1} (n = 53), than African scops owls 23.01 (± 7.24) ng g^{-1} (n = 12).

Behaviour in absence of customers

As the focal sampling was conducted only when there were no customers in the cafe, there was a difference in the total observation time per owl, ranging from 57 to 111 min (Table 1). The percentage of observed time categorised as each type of behaviour varied with individual owl (Figure 2). Overall, little negative behaviour was recorded, but it was most common in Owls 5, 6 and 8. Resting behaviours and/or neutral behaviours occupied the largest proportion of time for all owls.

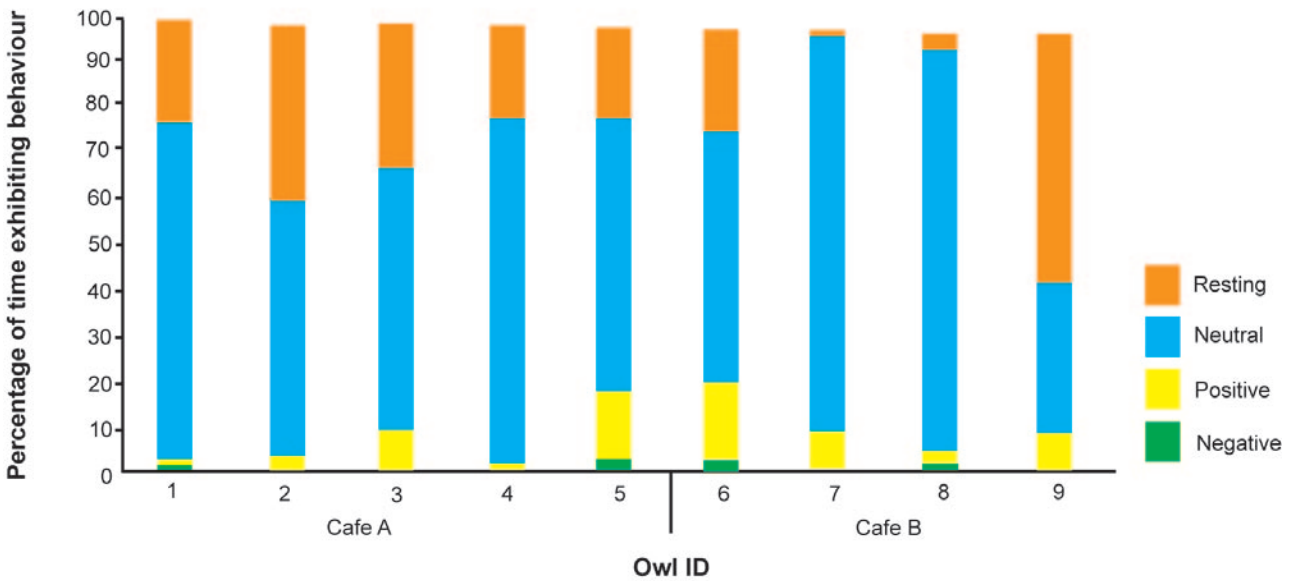
Customer interactions

The number of people interacting with each owl on a given day varied from 0 to 176 and averaged 46.5. The number of individual customer interaction events received (NIE) differed between owls, with the average being 35.6 per day but ranging up to 95. Although three owls were never lifted, for the remainder there was a mean of 3.1 and a maximum of eight carrying events per day. In response to the interactions, no affiliative (PAfR) reactions were observed in the owls and the percentage of neutral (PNR) and aversive (PAvR) reactions varied between owls (Figure 3) with Owls 1, 2 and 5 showing the highest PAvR and owls in cafe A (Owls 1–5) generally showed higher PAvR than Owls 6–9 (cafe B).

Hypothesis testing

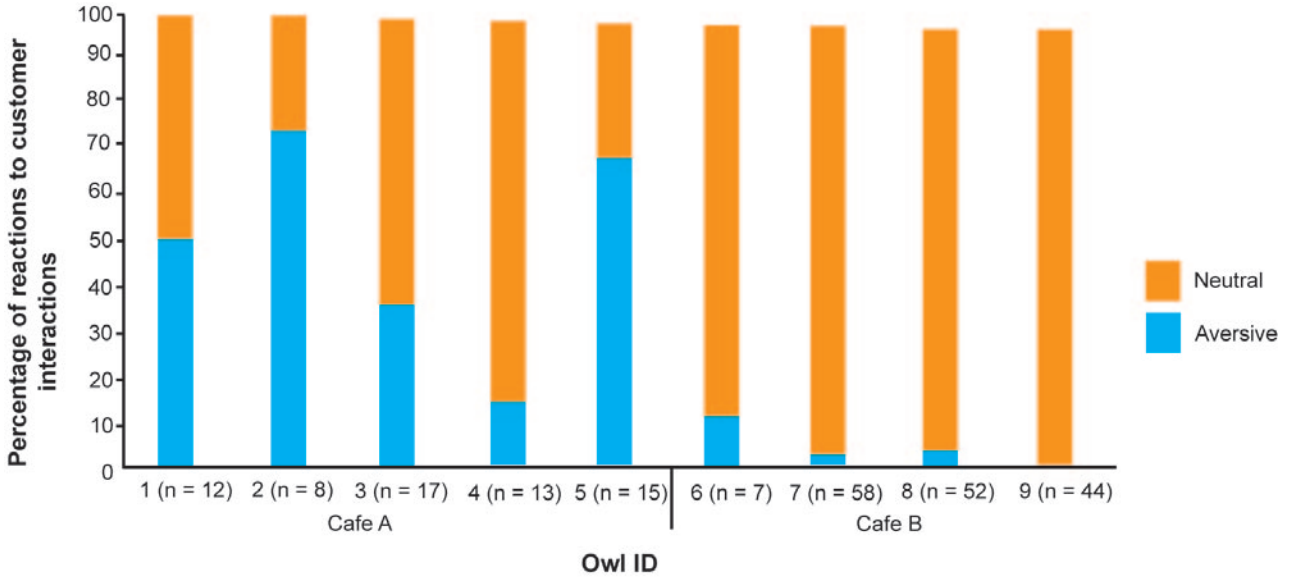
Univariate GLM tests revealed significant differences in FCL between individual owls ($F_{4,42} = 15.2$; $P < 0.001$) and between open and closed days ($F_{1,42} = 16.8$; $P < 0.001$); with mean levels being significantly higher on open (61.8 [± 48.73]) as compared to closed (35.6 [± 14.70]) days (Figure 4); and all four owls with adequate samples having higher levels on open as compared to closed days. The interaction between owl and open status, was not significant ($F_{3,42} = 0.2$; $P = 0.88$).

Figure 2



Percentage of time in each behavioural category for each owl (see Table 3 for definition of behavioural categories).

Figure 3



Percentage of aversive and neutral reactions to customer interactions for each owl. No affiliative reactions were observed.

When comparing individual owls' FCL to their undisturbed behaviour, no significant correlation was found with either Proportion of Negative Behaviours (PNB; Rho = 0.14; $P = 0.72$) or Proportion of Positive Behaviours (PPB; Rho = 0.07; $P = 0.87$).

Similarly, when looking at the owls' reactions to customer interaction, the association between FCL and PAvR was non-significant (Rho = 0.53; $P = 0.14$). However, the small sample size (n = 9), and the marginal result suggest that if the sample number were larger, this correlation may have been significant, especially for eagle owls. For example, Owl 2 shows high average FCL and high PNR, and Owl 8 shows low FCL and PNR (Figure 5).

When comparing between days, no significant relationship was found between FCL and any of the measures of customer level (NCP: $F_{1,48} = 0.40$; $P = 0.53$; NCI: $F_{1,48} = 0.33$; $P = 0.57$; NIE: $F_{1,48} = 0.57$; $P = 0.45$; NCE: $F_{1,30} = 0.97$; $P = 0.33$).

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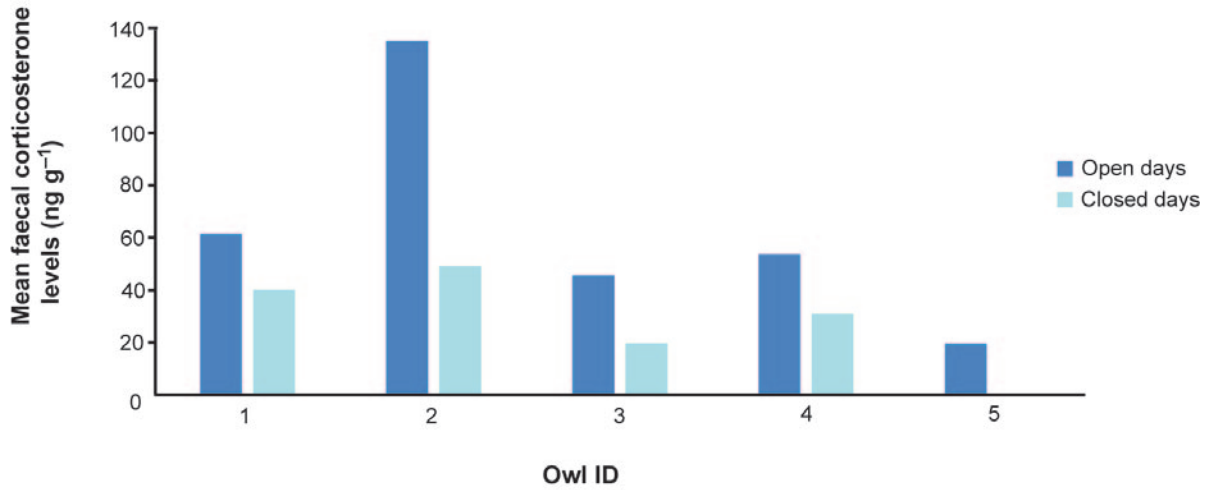
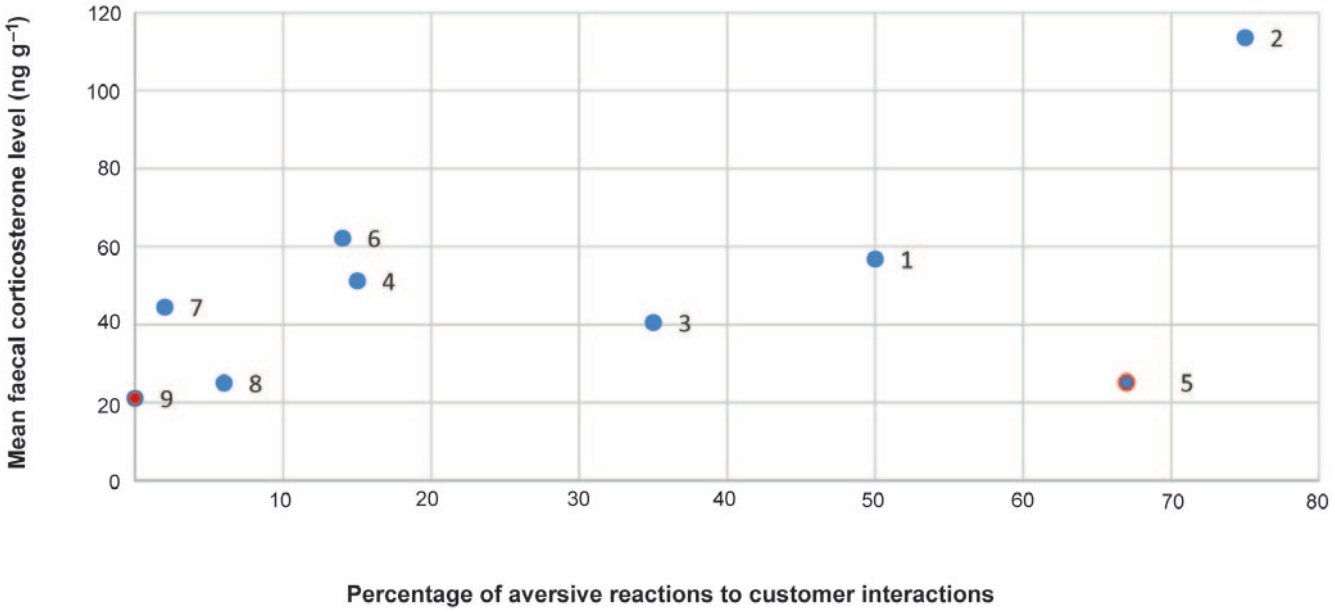
Figure 4



Mean faecal corticosterone levels (ng g^{-1}) for open and closed days in cafe A. Owl 5 has one missing value due to a lack of defaecation on a closed day.

Figure 5



Correlation between mean FCL and proportion of negative reactions to customer interactions (numbers correspond to owl ID).

Discussion

Owls were on view to customers in each of the cafes for 7 h per day, during which they were subject to multiple approaches and interactions from customers. The two cafes varied in terms of numbers with B having as many as 95 customers being present and approaching owls on any given day and individual owls being interacted with up to 59 times, thereby creating a significant potential for impact to the owls.

When observed undisturbed, the owls generally showed low levels of behaviours which could be described to be negative, but also low levels of positive behaviours such as preening. Here, we categorised agitated alert, as a negative behaviour, since enhanced vigilance can be due to the animal feeling threatened, although general alertness also indicates curiosity (Beauchamp *et al* 2019). Further study of subtle differences in alert states and optimal levels in owls are still required. Neutral and resting behaviours were the most common which is unsurprising given daytime is naturally the owls' resting time. However, chronic stress can potentially affect sleep and rest behaviours, and inactivity

can be a sign of compromised welfare (eg Fureix & Meagher 2016), so future comparison of the 24-h time budget of owls in cafes compared to the wild would be valuable. When approached by customers, owls were never observed to approach or show affiliative behaviours and, for several owls in cafe A, the responses were predominately aversive, such as withdrawing.

Individual owls varied significantly in mean FCL and African scops owls were significantly lower than eagle owls. Since FCL increases rapidly (within hours) after a stressor, faecal corticosterone reflects the integration of all adrenal activity in a given time-frame, including basal function and acute responses. The difference between the two cafes was not significant, but within cafe A, as hypothesised, FCL on open days was significantly higher than on closed days, suggesting that something in the routine when the cafe was open was more arousing and potentially stressful to the owls, compared to days when the cafe was closed. However, cafe A (which had closed days and considerably fewer customers per day) had owls with generally higher FCL suggesting that including closed days in the schedules is insufficient to reduce stress, since other environmental aspects play a role in determining FCL. The difference between the cafes compared was small (both were open for 7 h per day, but A had one closed day per week while B was open every day) and therefore greater disparities in the data might have been seen had we compared cafes with a wider variation of opening times.

We hypothesised a positive correlation between an individual owl's FCL and the Percentage of Negative Behaviours (PNB) displayed when undisturbed and a negative correlation between an individual owl's FCL and Percentage Positive Behaviours (PPB). This was not supported, as the behaviour of individual owls in the absence of customers showed no significant relationship to their FCL, suggesting that owls' behaviour in the absence of customers is not a significant predictor of stress level. There was, however, a tendency for owls showing a higher percentage of aversive reactions in response to customer interactions, to also have higher corticosterone levels (Rho = 0.53; $P = 0.14$). It is possible that this relationship may have reached significance had the sample size been bigger. Those owls which showed higher levels of aversive reactions during human interaction tended to present higher levels of physiological stress, suggesting that the owls' perception of interactions with humans may be an important determinant of their stress level. When examining the past history of the birds, there were no obvious predictors of behaviour or corticosterone apart from the fact that Owls 5, 6 and 8 (which showed the highest FCL) tended to be younger birds. This may be due to owls habituating to the cafe environment as they age or FCL reducing following repeated arousal.

The measures of customer intensity (NCP, NCI, NIE and NCE) did not show significant association with FCL, as had been hypothesised, so days with an increased number of people present or interacting with the owls did not result in an increase in stress. There are several possible explanations for this. Factors associated with the cafe being open other than the number of customer interactions may determine owl stress levels, for example, light levels in the room and the relative positioning of the owls. Lighting in the room where the owls were kept was turned off on closed days, apart from when the staff fed the birds. Owls are nocturnal, and most nocturnal species function best at dusk and dawn (Martin 1990). Hence, interactions with customers in a bright room may be stressful. Locations in which owls were tethered also differed on open days, and perhaps the owls preferred where they were tethered on closed days. The presence of unfamiliar humans could itself be a stressor to the owls, regardless of the interaction levels. It is possible that the owls recognise open and closed days and the expectation of customer interactions on open days is sufficient to raise general stress levels, irrespective of the number of interactions. Finally, there is a possibility that owls experience differential stress depending on the type of interaction (eg whether there is physical contact and whether it entails rough or gentle handling), and characteristics of the customers such as their height, odour, familiarity, and behaviour, rather than simply the number of interactions. We did not record the nature of the customers' behaviour, merely whether they picked the bird up, and subtler differences should be recorded and examined in future studies. Additional factors, such as individual owls' previous experiences of customers, their housing, lighting and consistency of tethering locations should also be explored. A greater understanding of which of these factors has the greatest influence on the owls' stress levels will help us derive meaningful recommendations to protect the welfare of owls in cafes in the future.

Differences between owls

While FCL showed a significant inter-species difference, for many samples from African scops owls, the amount of corticosterone was too low to quantify. A significant difference in mean FCL was found between owls with individuals 1, 2, and 6 showing particularly high levels. According to the cafe owner, Owl 1 was an escaped pet adopted by the cafe while Owl 2 had been kept as a pet for over a year but was brought to the cafe the year of data collection when its owner became ill. Owls 1 and 2, therefore, were not habituated to humans from an early age and they also showed higher Percentage Aversive Reactions than most other owls that came to the cafe as chicks. However, Owl 6 came to the cafe as a chick but still avoided customer interactions. Owl 6 was not tethered during opening hours and always stayed on a perch high above ground where customers could not touch it, showing a strong preference for not interacting with customers. While the owl did not show high PNR, the number of customer interactions was extremely low compared to other owls due to this pattern of avoidance. This illustrates how individuals can vary in the extent to which they find the environment aversive and further studies to identify predictors of calm owls on a larger sample size are now needed.

Although a small initial study, this work provides the first introductory examination of the potential welfare issues asso-

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ciated with Japanese owl cafes. The use of corticosterone as an indicator of stress seems promising in owls, but requires caution. The finding that owls with higher FCL tended to exhibit higher PAvR supports the potential value of FCL. Further studies should continue to use behaviour as well as physiological indicators of welfare and, ideally, examine FCL several hours subsequent to behavioural recordings to reflect corticosterone secreted over the entire day in the cafe.

Such a short-term study cannot account for environmental factors that can have an effect on animals and which may change over time (eg season) and so future studies would benefit from including data from a greater number of cafes and over a longer period as well as varying times of year. They should also consider 24-h observations and measures of stress, to assess how the owls behave when the visitors leave. It is important to acknowledge that our study was naturalistic and observational so further experiments imposing treatments (eg opening vs closed), randomly allocated to a larger number of owls are required to confirm the results of this preliminary study. Furthermore, an experiment in which human behaviour, approach and interaction was controlled to assess the owls' reaction and preference for customer interactions of both a positive and negative nature would complement this work.

Animal welfare implications and conclusion

This study investigated the effects on the welfare of owls in owl cafes when these were open vs closed, as well as the influence of the level of human interactions. We found that FCL levels on open days were significantly higher than on closed days, suggesting that open days are more stressful for the owls. Furthermore, while the correlations between FCL and both undisturbed behaviour (Proportion of Negative and Positive Behaviours) and level of customer interactions (NCP, NCI, NIE and NCE) were non-significant, there was a tendency for owls with higher PAvR (Percentage Aversive Reactions) to show higher FCL suggesting that customers may be a significant stressor for individual owls. To improve owl welfare in cafes in the future, we need to determine which factors most affect FCL (and hence result in the increase on open days) and the likelihood of owls showing aversive reactions to people. Potential factors include customer behaviour, environmental factors (such as light levels and owl positioning) or the 'sense of security' resulting from recognition of closed days. This knowledge could help identify modifiable environmental factors which could be altered to mitigate stress for the owls, for example, reducing light levels. However, if the most important factors include customer interactions it could be argued that owls are not suited to facilities such as owl cafes — or, at least, that owls with high FCL and high PNR (such as Owls 1 and 2) should not be kept in owl cafes. There are numerous factors in the cafe environment which may be aversive, and which may impact upon the owls' five welfare needs (Animal Welfare Act 2006), ranging from lack of constant access to water to an inability to perform natural flying behaviours. This study suggests that they may be stressful, however a fuller audit and assessment, making use of the animal welfare assessment grid (Justice *et al* 2017) would now be very valuable.

Declaration of interest

None.

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