

Stress as an adaptation I: Stress hormones are correlated with optimal foraging behaviour of gerbils under the risk of predation

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ABSTRACT

Background: Many organisms live in a world awash with predation risk. Optimal foragers trade off food and safety to maximize their fitness. A way for organisms to modify their behaviour, and appropriately trade off food and safety under changing conditions, is to do so as a function of stress hormone (glucocorticoid) concentration.

Hypothesis: As the risk of predation changes, stress hormones and an organism's optimal foraging behaviour will change accordingly.

Methods: We evaluated connections between a stress hormone – faecal glucocorticoid concentration (FGC) – and optimal foraging behaviour (using the giving-up density technique) in two species of desert gerbils, *Gerbillus andersoni allenbyi* and *Gerbillus nanus*, in a large outdoor enclosure. Gerbils were subject to changing predation risk from barn owls (*Tyto alba*, present or absent) and moon phase (nights with a full moon being riskier).

Results: Gerbils had higher giving-up densities (foraged less) and were more apprehensive (a form of vigilance) on nights with a full moon and when owls were present. Also, gerbils showed elevated FGCs in response to the full moon. Owl presence or absence, however, was not related to FGC. Individual gerbils with higher FGC foraged longer, ate more food, and foraged later into the night. Hence, in this system there is a correlation between optimal foraging under the risk of predation and stress hormones. Stress hormone concentrations increase in response to FGC, an indicator of general predation risk.

Keywords: desert rodents, faecal glucocorticoids, gerbils, moon phase, optimal foraging, predation risk, quitting harvest rate.

INTRODUCTION

For most organisms, predation risk varies in both space and time. The need to trade off food and safety, varying levels of available resources, the intensity of competition, as well as the comings and goings of predators, all mean that foragers must constantly reassess risk and their own energetic state, and adjust the relative value that they place on food and safety (Lima and Dill, 1990; Lima, 1998; Kotler *et al.*, 2004a). Such reassessments are reflected in the behaviour of the forager. But what is the physiological mechanism that leads to this readjustment?

Changes in stress hormone concentrations may be one avenue through which foragers alter their perception of risk and the value of energy. Glucocorticoid hormone (i.e. stress hormone) production, considered to be a reaction to stress, is often interpreted in a negative biological context. But it may largely represent an adaptive reaction (Carr and Summers, 2002; Wingfield and Kitaysky, 2002; Wielebnowski, 2003). For example, accumulating experimental evidence in birds and mammals indicates that glucocorticoids have a number of behavioural and physiological effects that promote fitness (Wingfield and Kitaysky, 2002; Jaatinen *et al.*, 2014).

The fact that foragers respond to predators is well documented (Lima, 1998). In the present study, we exposed gerbils to owls and to different moon phases (more moonlight results in greater risk for nocturnal gerbils). Similar studies (Kotler *et al.*, 1994; Abramsky *et al.*, 1996; St. Juliana *et al.*, 2011) have shown that gerbils, including those species used in this study (Allenby's gerbil, *Gerbillus andersoni* and the Baluchistan gerbil, *Gerbillus nanus*), respond to owls, snakes, and changes in moonlight in a functionally adaptive manner. Here, we expand on this body of research by linking the optimal foraging behaviour of the prey animals to stress hormones.

Evidence from many species suggests that predation risk may alter stress hormone concentrations in animals, including rodents (Eilam *et al.*, 1999; Harper and Austad, 2000; Bamberg *et al.*, 2001; Hayssen *et al.*, 2002; Touma *et al.*, 2003; St. Juliana, 2005). For example, in pied flycatchers (Silverin, 1998) and great tits (Cockrem and Silverin, 2002), glucocorticoid concentrations increased in response to the presence of a stuffed predator that moved. Similarly, when presented with a variety of stressors, stonechats exhibited the greatest glucocorticoid response to an actual predator (Canoine *et al.*, 2002). Mateo (2007) demonstrated a potential link between anti-predator behaviour and stress hormones in Belding's ground squirrels. Predation risk is also implicated in increased glucocorticoid concentration in the snowshoe hare (Boonstra *et al.*, 1998). Finally, Eilam *et al.* (1999) found that voles reacted to predator vocalizations (owl calls) through increased glucocorticoid concentration and an increased freezing or fleeing response.

Stress hormones have also been implicated in the regulation of feeding, namely stress hormones are usually associated with increased food intake (Adam and Epel, 2007). However, glucocorticoids appear to stimulate behaviours depending on context, including feeding, fighting, searching, running, and risk assessment (reviewed by Dallman *et al.*, 2007). Moreover, hunger by itself can cause an increase in stress hormone concentrations (e.g. Medhamurthy *et al.*, 2007). Therefore, we expect glucocorticoids to influence the allocation of time and energy to foraging behaviours.

Stress hormones may be one mechanism by which animals modulate behaviour adaptively to maximize fitness. Wingfield *et al.* (1998) presented a model based on an energetic currency that links stress hormones and foraging. Similarly, Hendrie *et al.* (1998) documented a variety of behavioural responses in rodents that likely influence their foraging ability. However, a variety of factors other than energy affect fitness and regulate the decision of an animal to move, seek refuge or forage. These include other fitness-enhancing activities

such as seeking mates, defending territory, and seeking safety (Brown, 1988, 1999; Brown *et al.*, 1999). Clinchy *et al.* (2004) argue that understanding trade-offs between food and safety is critical to our understanding of the relationship between stress hormones and behaviour.

Optimal foraging theory presents us with an excellent framework to link trade-offs and behaviours that are closely tied to fitness with stress hormones. Consider when a forager should leave a given resource patch in a world full of predators, where the forager has alternative activities other than foraging, and where its foraging activity – and that of others – can lead to resource depletion. Foraging theory provides a solution (Brown, 1988, 1992). An optimal forager should exploit a food patch so long as its harvest rate of resources from the patch (H , the harvest rate) exceeds its energetic (C), predation (P), and missed opportunity costs (MOC) for foraging, i.e. a forager should quit a patch when $H = C + P + MOC$. To address the links between hormones and behaviour, we used the concept of the giving-up density (GUD) from foraging theory (Brown, 1988, 1992). The GUD is the density of resources left behind when an organism quits foraging in a patch. (It is proportional to the quitting harvest rate.) For an animal exploiting a food patch in which it depletes the available food and suffers a corresponding reduction in harvest rate [i.e. diminishing returns (Kotler and Brown, 1990)], the GUD reflects foraging efficiency and can be used to measure harvest rate and various costs of foraging (Brown, 1988). Animals typically respond to increased risk of predation brought about by the presence of a predator by increasing their GUDs (Kotler *et al.*, 2004a; St. Juliana *et al.*, 2011).

Balancing food and safety affects foraging behaviour because organisms can use time allocation (Brown, 1999; Brown *et al.*, 1999) and apprehension (Dall *et al.*, 2001; Kotler *et al.*, 2002) to manage risk. *Time allocation* sets the where, the when, and the quantity of time devoted to foraging, while *apprehension* is the redirection of attention to other activities that reduce the probability of injury or death (such as predator detection). But apprehension reduces foraging efficiency (e.g. reduced harvest rate at a given resource density). The reduction in foraging efficiency can be a result of either overt vigilance or a diminished ability to assess patch boundaries, handle food items, and harvest food (Dall *et al.*, 2001). The ‘anxiety response’, so often studied in rodents (Adamec and Shallow, 1993; Blanchard *et al.*, 1995, 1998; Hendrie *et al.*, 1997; Belzung *et al.*, 2001), may reflect apprehension and could be a mechanism for regulating time allocation and apprehension. The latter two behaviours, in turn, can be correlated with stress hormone concentrations.

Animals could also vary their harvest strategy in response to predation and competition. Anderson’s gerbils are known to harvest seeds in two ways. They can grab and go (GAG), quickly filling their mouths with food and carrying it elsewhere to consume or cache; or they can eat at tray (EAT), eating the resources where they are found. As GAG foraging results in a higher harvest rate for *G. a. allenbyi* (Ovadia *et al.*, 2001), we predicted that when predation risk is higher, gerbils will benefit from using the more efficient (GAG) harvest strategy that reduces their time exposed to predators. A disadvantage of GAG foraging can be that gerbils may need to make many trips to and from the resource patch.

Hormones might be critical in allowing animals to trade off food and safety in the appropriate manner as circumstances change. How then is stress – as indicated by an increase in hormone concentration – related to changes in foraging behaviour in response to alterations in predation risk?

We manipulated predation risk by allowing gerbils to forage in the presence or absence of an owl, and during nights on which there was a new moon (less risky) or full moon (more risky) (Kotler *et al.*, 1991). We measured both the responses of individual gerbils and those

of the aggregate population to which they belonged. At the population (foraging tray) level, we predicted that increasing the risk of predation would lead to less time spent foraging, greater apprehension, less EAT foraging, higher GUDs, and higher stress (as indicated by faecal glucocorticoid metabolites). At the level of the individual gerbil, the relationship of foraging behaviour and stress hormones is not so easy to predict. Stress hormones (as indicated by faecal glucocorticoid metabolites) could be related either positively or negatively to foraging behaviours, depending on whether the hormones are cause or effect.

If stress hormone concentrations increase in the presence of increased predation risk (i.e. effect), they ought to rise in response to more overall foraging. Alternatively, suppose stress hormone concentrations play a causal role in an animal's decision-making (i.e. an increase in stress hormones leads to less overall foraging). Previous research has revealed a positive correlation between individual gerbil foraging effort and faecal stress hormone concentrations (St. Juliana *et al.*, in review b). Thus, we predicted that, at the individual level, individuals with higher circulating concentrations of stress hormones would: (1) allocate more time to foraging, (2) consume more food, (3) have lower GUDs, (4) start foraging sooner, and (5) finish foraging later.

METHODS

Enclosure methods

We used two species of desert gerbils, *Gerbillus andersoni allenbyi* (30 g) and *Gerbillus nanus* (11 g), in our study. Study animals were caught wild in the field.

We conducted the experiments in a large outdoor enclosure measuring 17×34 m divided into four sections, each measuring 8.5×17 m. We maintained a density of ~4 gerbils in each section throughout the study. When adding gerbils to a section, we always did so in a 1:1 sex ratio (or as close to this ratio as possible). We marked each individual rodent with a uniquely numbered PIT (passive induction transponder) electronic tag. Each section of the enclosure had four stations. Each station had a low trellis on which we placed cut brush to simulate a shrub. The gerbils could forage from a seed tray under this artificial shrub. Each plastic seed tray ($28 \times 38 \times 8$ cm) contained 3 g of millet seed mixed into 3 litres of sand. Below each tray, we placed a PIT tag reader attached to a data logger.

We ran this experiment twice: once with *G. nanus* in two sections and *G. a. allenbyi* in two sections (19 April to 17 May 2010), and a second time with *G. a. allenbyi* in all four sections (13 October to 10 November 2010). Before the start of each run, we gave the gerbils 6 days to acclimate to the conditions in the enclosures.

We devised predator and no-predator treatments. In the predator treatment, two barn owls (*Tyto alba*) were allowed to fly around the experimental arena. The owls were allowed to catch and eat the gerbils. We looked for pellets in the owls' daytime enclosure and data from the tag readers to detect gerbil deaths. The treatments occurred in blocks of eight nights each, arranged around either the new moon or full moon (four nights with owls and four nights without). These moon phases represented a second level of predation risk because nights on which there is a full moon are riskier for gerbils than nights with a new moon. After the fourth night of a given predator treatment in a moon phase, we switched treatments. Each group of animals therefore experienced the owl and no-owl treatments twice (once at each moon phase). In total, there were 32 experimental nights.

On each night of the experiment, we collected behavioural data at the tray level, which is an aggregate measurement of all the individuals that foraged at a particular tray. Following each experimental night, we sieved the sand of each tray to remove remaining seeds, and then took those seeds to the laboratory to be cleaned of debris and weighed, thus obtaining the GUD for each tray. The trays were equipped with PIT tag readers to record (1) individual forager identity, (2) the time, and (3) the duration of every visit to the tray. Using the GUDs and the total time individuals foraged in trays, we generated a harvest curve from which we could look at residuals to get a measure of apprehension. A negative residual represents a lower GUD for a given time allocation and also represents greater apprehension. We also collected the discarded seed husks from each tray, which enabled us to measure the proportion of EAT foraging used at that tray.

We were able to ascertain the following for individual gerbils: average quitting harvest rate (for all trays foraged in for more than 90 seconds), total time foraging in all trays, time at start of forage, time at end of last forage, and total food consumed.

Every second night, we opened Sherman traps set alongside the feeding trays one hour before dawn to capture animals for obtaining faecal samples for quantifying stress hormones. Four hours after the traps were opened, we released any captured animals from the traps and collected their faeces for hormone analysis. Due to the previously established time lag of steroid metabolism, the faeces collected at this time are representative of circulating stress hormones that circulated during the night while the animal was still foraging rather than while in the trap (St. Juliana *et al.*, in review a).

For the first trial of the experiment, we collected GUDs and time foraging every night, but only %EAT foraging on the nights when gerbils were trapped. For the second trial, we collected time foraging every night, but only GUDs along with time foraging on nights when we trapped gerbils. Any tray that was not foraged was excluded from the GUD analysis. On several occasions, the tag reader under a tray failed to function; these points were excluded from the analysis.

Faecal collection and processing

We collected faeces to measure faecal glucocorticoid metabolite (FGM) concentrations. Faeces were collected from the traps no later than 2 hours after the animal was released. Any faeces contaminated with urine were excluded from analysis. The faeces were placed in a tin foil bag and immediately frozen at -32°C . After the last experimental round, all foil bags were soaked in liquid nitrogen for 30 seconds and then freeze-dried for 24 hours. This procedure facilitated transportation and inhibited bacterial growth. We processed the samples at the endocrinology lab at the Chicago Zoological Society (Brookfield Zoo, Brookfield, IL, USA). After arrival at the lab, the faecal samples were stored in a -20°C freezer. They were processed by first placing them on a piece of weigh paper. The weigh paper was then folded in half and the faeces in the middle were pulverized with a rubber mallet. The faeces were then placed in a plastic test tube in a freezer for 5–8 days prior to being weighed. Before weighing, the faecal sample in the test tube was vortexed. (This treatment helped to further break up the sample.)

To extract steroid metabolites, 3 mL of 80% ethanol was added to the dry faecal material in polyethylene tubes. Tubes were placed on a rotator overnight and then centrifuged for 15 minutes at 1500 rpm. One millilitre of supernatant was removed and diluted with 1 mL assay buffer [0.1 M phosphate-buffered saline (PBS) containing 1% BSA, pH 7.0].

We used a commercially available corticosterone EIA kit from Assay Designs (Ann Arbor, MI, catalogue # ADI-901-097) to analyse faecal samples for FGM concentrations, following the manufacturer's instructions. Plates for the EIA were read on a photo-spectrometer plate reader (Dynex MRX Revelation, Dynex Technologies, Chantilly, VA) at 405 nm. The cross-reactivity of the corticosterone antibody is as follows: 100% corticosterone, 28.6% desoxycorticosterone, 1.7% progesterone, 0.28% tetrahydrocorticosterone, 0.18% aldosterone, 0.13% testosterone, 0.046% cortisol, and any other steroids <0.03%.

The assay that we used was validated both biochemically and biologically before beginning our study. The sensitivity of the assay was $26.99 \text{ pg} \cdot \text{mL}^{-1}$, and the intra-assay coefficient of variation was 4.94% at 58.39% binding, 6.86% at 56.01% binding, and 3.76% at 32.1% binding. Recovery of exogenous corticosterone ($250\text{--}4000 \text{ pg} \cdot \text{mL}^{-1}$) was 74.91%. To validate the assay biologically, we conducted an ACTH challenge on both of the species used in this study (St. Juliana *et al.*, in review a). For *G. a. allenbyi* ($n = 4$), there was a peak in FGM concentration after 9.25 hours on average, with an average percentage increase of 368% from the baseline value. For *G. nanus* ($n = 4$), we observed a peak in FGM concentrations after 6.25 hours on average, with an average percentage increase of 308% from baseline. FGM concentrations are presented as nanograms per gram dry weight of faeces.

Data analyses

For the analysis of tray foraging, we ran standard least squares mixed models that included moon phase (full or new), predator (owl or no owl), and the interaction of moon phase and predator as independent variables. In each model, species (*G. a. allenbyi* or *G. nanus*) was included as a random factor (because we had no predictions as regards species). Tray ($n = 16$) nested within section ($n = 4$) was also included as a random factor. The dependent variables for these analyses were: GUDs ($n = 383$), apprehension ($n = 346$), %EAT foraging ($n = 251$), and time foraging in each tray ($n = 439$). The GUD values were $\log_{10}$ -transformed to meet the assumptions of normality; % EAT was arc-sin-square-root transformed for normality.

To assess the influence of predation on FGM concentrations, we averaged any individual hormone values that occurred more than once in a treatment. For example, if a gerbil was caught on both of the new moon/no-owl nights, we averaged its hormone values. This resulted in 93 hormone-concentration data points. We ran a standard least squares mixed model with faecal glucocorticoid concentration per gram of faeces as the dependent variable and moon phase (full or new), predator (owl or no owl), and the interaction of moon phase and owl as independent variables. Species was included as a random factor. Hormone values were $\log_{10}$ -transformed to meet the assumptions of normality.

We conducted an analysis relating an individual's faecal glucocorticoid concentrations at the level of the individual. We averaged the hormone concentrations and each aspect of behaviour for the entire experiment for each individual. Forty-one animals were included in this experiment, but four were not trapped, resulting in 37 individuals for which we had both hormones and the different aspects of behaviour. We ran standard least squares mixed models that included faecal glucocorticoid concentration as the independent variable and species as a random factor. The dependent variables in these analyses were quitting harvest rate, foraging start time, foraging end, total time foraging, and total food consumed.

All random effect values are for two-tailed tests. We do not discuss any significant random factors, as they were not used to make predictions but instead were used to account for variance due to differences between species and sections/trays.

RESULTS

Tray-level behavioural results

The GUDs varied as a function of both moon phase and owl presence or absence (Table 1a, Fig. 1a). Gerbils had lower GUDs on nights with a new moon and higher GUDs when an

Table 1. Standard least squares mixed-model fixed effects statistics for the influence of owls and moon phase on tray-level behaviours: (a) giving-up density (GUD), (b) apprehension, (c) proportion EAT (eat-at-tray) foraging, and (d) time allocated to foraging

Source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
(a) Log GUD				
Moon	1	22.6944	169.9263	< 0.0001*
Owl	1	1.9621	8.535	0.0019*
Moon*Owl	1	1.46733	10.4695	0.0007*
Species (Random)	1	0.4129	2.9462	0.8437
Tray in section (Random)	12	0.0839	0.5982	0.0869
Error	366	0.1402		
(b) Apprehension				
Moon	1	2.7342	26.8509	< 0.0001*
Owl	1	0.3674	3.6086	0.0292*
Moon*Owl	1	0.4596	4.5135	0.0172*
Species (Random)	1	0.0569	0.5583	0.4555
Tray in section (Random)	12	0.4896	4.8057	< 0.0001
Error	329	0.1018		
(c) Proportion EAT				
Moon	1	0.8127	6.932	0.0046*
Owl	1	0.5532	4.7131	0.0172*
Moon*Owl	1	0.0198	0.1688	0.3408
Species (Random)	1	7.8149	66.5841	0.0001
Tray in section (Random)	12	0.1444	1.2305	0.2628
Error	234	0.1174		
(d) Time allocated to foraging				
Moon	1	239939383	72.1100	< 0.0001*
Owl	1	13501.52	0.0041	0.4357
Moon*Owl	1	27419264	8.2404	0.0022*
Species (Random)	1	111436247	33.4904	< 0.0001
Tray in section (Random)	12	23354380	7.0188	< 0.0001
Error	422	3327409		

Note: See Methods for details.

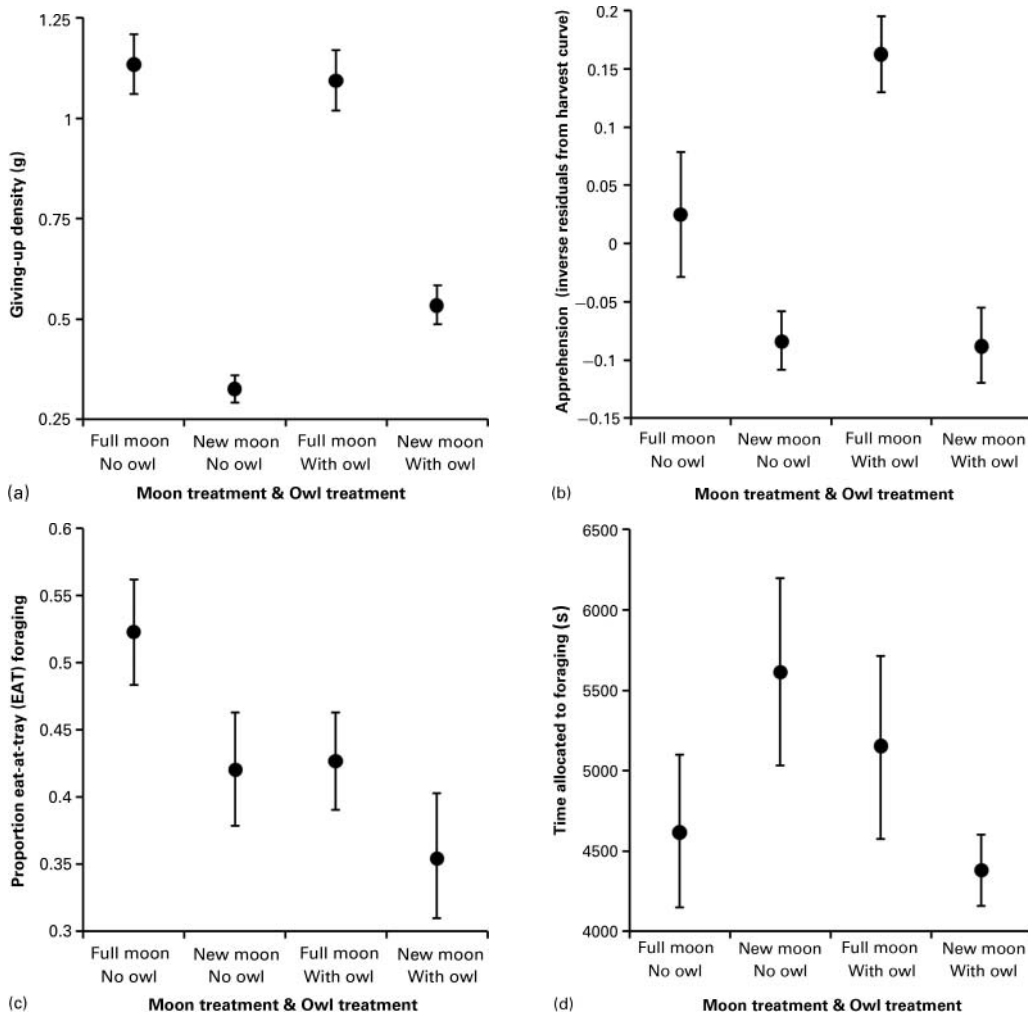


Fig. 1. The influence of owls and moon phase on selected gerbil behaviours: (a) giving-up density (GUD), (b) apprehension, (c) proportion EAT (eat-at-tray) foraging, and (d) time allocated to foraging. Error bars represent standard error. Non-transformed values are presented for clarity. See results for details.

owl was present. There was a significant interaction term. On nights with a full moon, there was no difference in GUDs between the owl and no-owl treatments. On nights with a new moon, gerbils had higher GUDs when owls were present.

Apprehension varied as a function of both moon phase and owl presence or absence (Table 1b, Fig. 1b). Gerbils were more apprehensive on nights with a full moon and when owls were present. There was a significant interaction term: on nights with a new moon, there was no difference in apprehension between the owl and no-owl treatments, whereas on nights with a full moon, gerbils were more apprehensive when owls were present.

The proportion of EAT foraging was higher during a full moon and lower when owls were not present (Table 1c, Fig. 1c). The interaction term was not significant.

Time allocation varied as a function of moon phase, but not owl presence or absence (Table 1d, Fig. 1d). Gerbils spent more time foraging on nights with a new moon. However, the presence/absence of an owl did not have a significant influence on time allocated to foraging. There was a significant interaction term such that on nights with a full moon, gerbils did not differ in time spent foraging, whereas on nights with a new moon, when owls were present the gerbils devoted less time to foraging.

FGM results

Faecal glucocorticoid metabolite (FGM) concentrations varied as a function of moon phase, but not predator presence/absence (Table 2, Fig. 2).

Table 2. Standard least squares mixed-model fixed effects statistics for the influence of owls and moon phase on faecal hormone concentrations

Source	<i>df</i>	<i>MS</i>	<i>F</i>	<i>P</i>
Moon	1	0.3481	6.3891	0.0067*
Owl	1	0.0277	0.5089	0.2388
Moon*Owl	1	0.0740	1.3579	0.1235
Species (Random)	1	0.3133	5.7493	0.0186
Error	88	0.0545		

Note: See Methods for details.

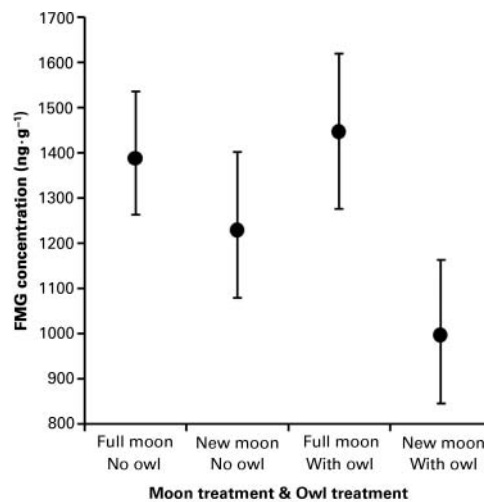


Fig. 2. The influence of owls and moon phase on faecal glucocorticoid concentration. Moon phase, but not owl presence/absence, influenced faecal glucocorticoid concentrations. Untransformed data are presented for clarity.

FGM concentrations were positively related to a gerbil's time spent foraging ($df = 1, 34.1$; $F = 5.1088$; $P = 0.0152$; $R^2 = 0.35$; Fig. 3a), total food consumed ($df = 1, 34.83$; $F = 4.2308$; $P = 0.0236$; $R^2 = 0.12$; Fig. 3b), and final time of foraging ($df = 1, 34.05$; $F = 5.6405$; $P = 0.0117$; $R^2 = 0.51$; Fig. 3c). In contrast, FGM concentrations were not related to a gerbil's quitting harvest rate ($df = 1, 34.1$; $F = 0.9462$; $P = 0.1688$; $R^2 = 0.28$) or start of foraging time ($df = 1, 34.15$; $F = 0.2097$; $P = 0.32495$; $R^2 = 0.22$). For time spent foraging and last time foraging, the trend largely reflects the behaviour of just a few *G. nanus* individuals (Fig. 3).

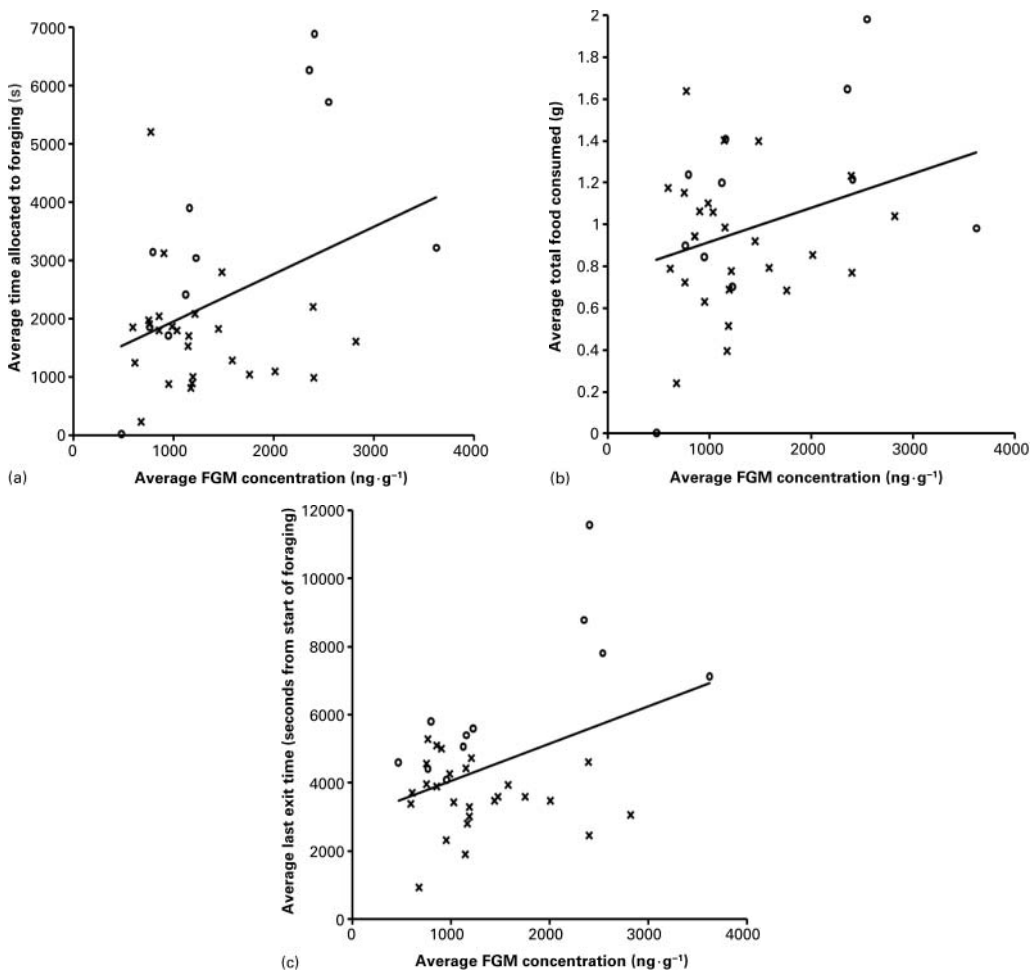


Fig. 3. The relationship between an individual's average faecal glucocorticoid concentration and the average of selected behaviours: (a) total time foraging, (b) amount of food consumed, (c) last foraging time. There is a positive correlation for all three variables. × = *Gerbillus andersoni allenbyi*, ○ = *Gerbillus nanus*.

DISCUSSION

Our results for the influence of moon phase and owl predation are mostly in line with other studies that have used this system (e.g. Kotler *et al.*, 1991, 2004b; St. Juliana *et al.*, 2011). Gerbils had higher GUDs during a full moon and when owls were present (Fig. 1a). However, GUDs when owls were present were higher only on nights with a new moon. It is possible that a full moon presented a strong enough risk, being an indicator of overall predation risk, to mask the influence of the owl. It is also possible that the predator treatment did not cause much perceived risk or actual risk for the gerbils. Gerbils were more apprehensive with a full moon and when an owl was present (Fig. 1b). They were similarly apprehensive on nights with a new moon when owls were present, and were most apprehensive when there was a full moon and owls were present. Although the gerbils used less eat-at-tray (EAT) foraging in the presence of owls, contrary to our prediction they performed more EAT foraging on nights with a full moon (Fig. 1c). One possible explanation for this result is that while 'grab and go' (GAG) foraging increases harvest rate in some circumstances, it may increase risk if the gerbils make many trips from foraging tray to cover or burrow on risky full-moon nights. In our experiment, all the foraging stations were under bushes, and foraging under a bush is likely safer than travelling to and from a burrow. Our time-allocated-to-foraging results showed that gerbils spent less time foraging on nights with a full moon (Fig. 1d). We did not detect an effect of foraging time in response to owl presence even though we did find a lower GUD when the owl was not present. This is possibly due to the gerbils' apprehension (Embar *et al.*, 2011). When an owl was present, they were more apprehensive but allocated a similar amount of time to foraging, and that combination produced a higher GUD. In other words, they had a lower harvest rate but foraged for a similar amount of time.

We observed higher FGM concentrations during nights with a full moon, but not in response to the presence of owls (Fig. 2). There are at least two ways to understand this result. First, the perceived predation risk caused by the owls irrespective of moonlight is lower than that caused by a full moon – in other words, the rodents deem being visible to owls to be risky, not just the presence of owls. Our tray-level results support this idea. Second, the consistency and predictability of risk may be a factor (Lima and Bednekoff, 1999). We know that the presence of a predator can cause increases in stress hormone concentrations of prey. Boonstra *et al.* (2014) suggest that stress hormones may function mostly as a pre-emptive mechanism as part of a longer-term adaptive response to the average or general predation risk of a given environment. In some situations, monthly or seasonal glucocorticoid (stress) hormone fluctuations in prey species may function to facilitate the crucial adaptive trade-off between food and safety under predictable and regular changes in predation risk (Gutman *et al.*, 2011; Kronfeld-Schor *et al.*, 2013).

As a function of increasing FGM concentration, gerbils increased the amount of time they allocated to foraging, consumed more food, and foraged later into the night (Fig. 3). However, FGM concentration was not associated with an earlier initiation of foraging or with quitting harvest rate (QHR). The QHR may not have shown a significant response because of the many assumptions that we made to calculate it. For example, we used the same harvest rate curve for all individuals and could not reliably know whether some foraging was just sampling of the trays by the animals. Perhaps we did not find an effect of start time because, to a large extent, all gerbils started foraging early. Early evening is when the food patches are at their richest and most animals, regardless of their energetic state, are

likely to forage in them because the high harvest rate they offer at that time outweighs the costs of foraging.

So how may stress hormones ultimately be related to predation risk in our model system? Typically, animals exposed to predation risk increase their defensive behaviours and also have higher stress hormone concentrations (Clinchy *et al.*, 2013; Liesenjohann *et al.*, 2013). Yet stress hormones are typically associated with increased (Dallman *et al.*, 2004), but sometimes decreased (Harris, 2015), feeding behaviour. Given the trade-off between food and safety, it is noteworthy that where food alone is concerned stress hormones are typically associated with eating more, which would imply taking more risk. But in studies involving predators, stress hormones are associated with eating less and taking less risk. In the case of the system used in the present study, FGM concentrations increased as a function of predation risk, but they also appear to cause animals to forage more on an individual level (Sargunaraj *et al.*, 2017; St. Juliana *et al.*, in preparation). This presents a conundrum for how stress hormones operate with regards to optimal foraging. It seems that in our system stress hormones do increase in response to predation risk, but they do not cause the animals to forage less. Instead, stress hormones may play a permissive role, leading animals to forage more when they otherwise might be too hesitant to do so.

Stress hormone fluctuations in accordance with predictable environmental indicators of predation risk may drive animals to trade off food and safety optimally. Unfortunately, although we could not measure an individual's apprehension directly, it is possible that stress hormones cause gerbils to forage more than they would have otherwise, but with increasing use of selected behaviours, such as apprehension/vigilance (Voellmy *et al.*, 2014; Sargunaraj *et al.*, 2017).

Ultimately, we were able to show that stress hormones are likely related to animal behaviour. They appear to be related to foraging time and energy acquisition. At the same time, FGM concentrations increase in response to moon phase, a slow changing factor that is associated with heightened risk of predation. Full moon is also a time of the month during which animals see their energetic state deteriorate due to a decline in the time in which it is safe to forage. Thus they may need to forage for longer than they otherwise might in order to prevent their energetic state deteriorating to dangerously low levels (Kotler *et al.*, 2010).

It would appear, then, that stress hormones may be driving some behaviours necessary for optimal foraging of prey animals that must trade off food and safety (Sargunaraj *et al.*, 2017). Our conclusions here are based on correlations between our experimental or environmental factors, the foraging behaviours of the gerbils, and their FGM concentrations. Thus the caveat must be raised of what is cause and what is effect? Sargunaraj *et al.* (2017) cut this Gordian knot by experimentally manipulating corticosteroids in Allenby's gerbils and exposing them to risk factors of moon phase and owls in the vivarium.

ACKNOWLEDGEMENTS

Funding was provided by the Israel Science Foundation, Research Grant Application #804/09 to B.P.K. This research was carried out with the approval of the Ben-Gurion University Committee for the Ethical Care and Use of Animals. We also thank the Israel Nature and National Parks Authority for granting us a research permit (#5-2-2011), and Dr Shira Ovadia, attending veterinarian, Ben-Gurion University of the Negev, for animal care and support. We thank Carola Bick for her assistance with the fieldwork, Jocelyn L. Bryant for her assistance with the lab work, and Karen Embar and

Sonny Bleicher for their general assistance with this experiment. Justin St. Juliana thanks Burt P. Kotler, Nadja Wielebnowski, Jonathan G. Cox, Chris Briggs, Cynthia J. Downs, and Paloma V. St. Juliana.

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