Testing the independent effects of population and shelter density on behavioural and corticosterone responses of tree skinks

Peter Lancaster[A], *Tim S. Jessop*[A,B,C] *and Devi Stuart-Fox*[A]

[A]Department of Zoology, University of Melbourne, Vic. 3010, Australia.
[B]Department of Wildlife Conservation and Science, Zoos Victoria, Vic. 3052, Australia.
[C]Corresponding author. Email: tjessop@unimelb.edu.au

Abstract.   In animals, social organisation and behaviour can respond to variation in key ecological factors including population and resource density. As these two factors covary, their relative importance is difficult to estimate using field studies. Consequently, we conducted two manipulative experiments varying levels of either population or shelter density to separate their effects on solitary, affiliative and agonistic behaviour and physiology in the social tree skink, *Egernia striolata*. We used focal observations and plasma concentrations of the hormone corticosterone to measure behavioural and physiological responses to these manipulations. Aggressive behaviours occurred more frequently at high skink density, with males at high density exhibiting social stress, as indicated by increased levels of corticosterone. Skinks at low densities showed greater affiliative behaviour, spending more time basking as pairs. Changes in shelter density influenced exploratory behaviours, with males at low shelter densities exploring enclosures more than those at high shelter densities. Skinks sheltered as pairs more frequently at low shelter density, even after taking into account differences in frequency of pair sheltering expected by chance alone, suggesting that low shelter availability promotes pair behaviour. Corticosterone levels increased over time at low shelter density, which may have been a result of thermal stress coupled with a lack of microclimate variation in comparison to high shelter density. These results suggest that population and resource density are key factors that can independently influence social behaviour and endocrinology, and consequently social organisation, in different ways.

Additional keywords: resource density, lizard, *Egernia striolata*, affiliative behaviour, aggressive behaviour.

Introduction

Ecological factors are important in the evolution of social behaviour, and potentially complex social organisation (Alexander 1974). Two important ecological factors that can induce changes in social behaviour are population density and resource density (Alexander 1974). Resources that are patchy in nature (e.g. shelter or food) will attract conspecifics to an area where resources are concentrated (Graves and Duvall 1995). Resource density and population density can influence an individual's frequency of affiliative, agonistic and solitary behaviours and can consequently influence social organisation (e.g. Judge and DeWaal 1997; Warburg 2000). As population density increases, the frequency of interactions among conspecifics increases (Warburg 2000), leading to more complex social behaviours and formation of stable groups. At high population density, social group formation is expected to be inhibited by increased agonistic behaviours as competition increases (Judge and DeWaal 1997). In contrast, increases in resource density reduce the need for conspecifics to share, potentially reducing the frequency of social interactions between conspecifics and, in turn, development of complex social organisation (Visagie *et al.* 2005). As these ecological factors covary (with resource density often influencing population density and *vice versa*) (Sutherland 1996), field studies on social organisation and behaviour are unable to partition their relative influences.

Lizards within the Australian genus *Egernia* have among the most complex social organisations in reptiles, making them ideal models for studying the evolution of sociality (Chapple 2003). Many *Egernia* species engage in long-term monogamy, pair bonding and delayed juvenile dispersal (Gardner *et al.* 2001; Chapple 2003; Fuller *et al.* 2005; While *et al.* 2009*a*). *Egernia* species show a wide range of social structures that are potentially influenced by ecological factors (Bull 2000; O'Connor and Shine 2003; Gregory 2004), including population and resource density. Specifically, environmental constraints such as shelter availability and habitat saturation (high population density) have been suggested to select for territoriality and delayed juvenile dispersal in *Egernia*, leading to the formation of family groups (Duffield and Bull 2002; Chapple 2003; O'Connor and Shine 2003; While *et al.* 2009*b*).

This study aimed to evaluate the influence of both population and resource density on the social behaviour and endocrinology in a lizard, the Australian tree skink, *Egernia striolata*. This species varies in its social structure (solitary versus social groups) depending on its habitat, suggesting that one or more ecological

factors are influencing the social organisation of this species. In woodlands it appears to be largely asocial and solitary (Bustard 1970; but see Derez 2004); however, in rocky outcrops in south-east Australia, it exhibits social groupings, often consisting of a monogamous breeding pair and related subadults and offspring (Bonnett 1999). Although it is possible that the rock- and tree-dwelling populations of this species represent separate genetic lineages, understanding the proximate ecological factors influencing social behaviours can provide insight into the origins of social behaviour and the variation in social behaviour that exists in *Egernia*. Key ecological characteristics that are likely to differ between the woodland and rocky outcrop habitats are population density and the type and density of shelters (Chapple 2003). We manipulated each of these factors during two separate, consecutive experiments conducted within outdoor enclosures. Experiment 1 comprised a high- and low-population-density treatment with the number of shelters per enclosure held constant while Experiment 2 comprised the converse (high- and low-shelter-density treatment with the number of individuals per enclosure held constant). We tested the effect of high- and low-density treatments in each experiment on solitary, affiliative and agonistic behaviour as well as circulating corticosterone levels. We specifically examined corticosterone levels as hormones often respond to and mediate changes in social behaviour caused by variation in ecological factors. For example, high population density can increase glucocorticoid stress hormones either due to increased activity associated with elevated aggression, particularly among males (i.e. fighting, threat displays and territorial patrolling), or more indirectly as a consequence of winner/loser effects (Rubenstein 2007).

In *Egernia*, low habitat availability due to limited dispersal among isolated rock outcrops and consequent habitat saturation has been hypothesised to promote social behaviour (Chapple 2003). Thus, we predicted that affiliative social behaviours should increase under high-population-density and low-shelter-density treatments. However, increased competition due to low habitat availability and/or high population density could also increase agonistic behaviour. In this situation, high population density may be expected to result in increased agonistic behaviours and, consequently, increased plasma corticosterone levels, particularly in males. However, plasma corticosterone is also expected to decrease over time as individuals form dominance hierarchies because winners (i.e. dominant animals) often have lower basal glucocorticoid levels in socially stable systems than losers (i.e. subordinate animals) (Keeney *et al.* 2006; Ostner *et al.* 2008), including in *Egernia* species (Chapple 2003). Finally, we predicted that plasma corticosterone concentrations should be lower in high-shelter-density treatments because increases in shelter density could potentially reduce endocrine stress responses by reducing exposure to ecological (e.g. predation) and biophysical (e.g. extremes of temperature) stressors (Rubenstein 2007).

Methods

Animal capture and husbandry

We captured 44 adult *E. striolata* in spring 2008 from the Mt Korong Nature Conservation Reserve in Victoria, Australia (36·460°S, 143·751°E). Here skinks were found at varying densities on rocky outcrops with lizards observed basking individually or in small groups. Skinks were hand captured using a fishing pole then measured for body size (snout–vent length, SVL) to the nearest millimetre and mass to the nearest 0.1 g. We sexed skinks by the presence (males) or absence (females) of hemipenes. Each skink was given a unique dorsal marking using a non-toxic paint pen to enable differentiation of individuals during behavioural observations. The mean size (SVL) of skinks used in the experiments was 107 mm, with size ranging from 98 mm to 118 mm (minimum SVL at sexual maturity = 90 mm: Bonnett 1999). Skinks were transported to Serendip Sanctuary, Lara, Victoria (37·99°S, 144·41°E), where behavioural experiments were conducted. Experiments started a week after capture of the last skinks as one week was sufficient for corticosterone levels to return to near baseline levels in *E. whitii* (Jones and Bell 2004). On average, the skinks had spent of 19 days (±7, s.e.) in captivity before experiments commenced.

Skinks were housed in outdoor wire enclosures (150 × 90 × 45 cm), ~1 m apart. To minimise visual contact between skinks in adjacent enclosures, we lined the bottom of each enclosure's walls with opaque plastic. Each enclosure contained 2–4 shelters, depending on the experimental treatment. Shelters consisted of cement tiles, 39 × 18 × 5 cm. Three tiles were piled to create a shelter, the bottom two piles separated by small wooden blocks, with the resultant gap replicating a natural crevice. Skinks were fed cat food, mealworms or crickets three times a week and water *ad libitum*. On average, skinks spent 19 days (±7, s.e.) in captivity to facilitate acclimation in captivity before experiments commenced (Jones and Bell 2004).

Experimental design

We conducted two separate experiments manipulating skink and shelter density respectively, allowing us to reuse enclosures and individuals, which were limited for logistical reasons. In Experiment 1, we manipulated skink density while keeping shelter density constant at two shelters per enclosure. The high-density treatment housed four skinks, two males and two females, and the low-density treatment contained one male and one female. There were seven and six replicates (enclosures) of the low- and high-density treatments respectively. Our field observations indicated that skinks were either solitary, or associated as male/female pairs, and that the minimum distances among individuals or pairs was several metres. Hence, the high-density treatment used here represents a substantial increase in density above that observed under our field conditions. We appreciate that other forms of density manipulation could be trialled here, including changing the sex ratio, or further increasing the number of individuals, which may lead to different behavioural or physiological results in this study. However, as a first attempt we have chosen a simple 2-fold increase in density while maintaining a constant sex ratio.

In Experiment 2, we manipulated shelter density while keeping skink density constant at two skinks (one male and one female). The high-shelter-density enclosures contained four shelters while the low-shelter-density enclosures contained two shelters. Low shelter density allowed skinks the option to shelter alone or together using the fewest number of shelters. Each treatment had 10 replicates.

Three days before commencement of each experiment, we arranged skinks in the experimental enclosures as described above, ensuring that individuals within enclosures were unfamiliar (i.e. we rearranged individuals before both Experiments 1 and 2). To minimise the possibility that behaviours were confounded by prior familiarity, relatedness or large differences in body size, which influence social interactions in this species (Cockerell 2000; Bull *et al.* 2001), we ensured that skinks in each enclosure were spatially independent (i.e. captured at least 50 m apart; mean estimated size of the home range of the tree skink is 45 m^2: Bonnett 1999) and of similar size and mass. Experiment 1 was conducted between 18 December 2008 and 6 January 2009, with Experiment 2 conducted between 10 January and 28 January 2009. These periods span the later part of the breeding season after mating in Spring (September–November) and prior parturition (February) (Chapple 2003). As females appeared to be gravid during the experiments, observed social behaviours are unlikely to simply reflect mating behaviours such as mate searching and mate choice.

Behavioural responses

To monitor changes in behaviour during both experiments, we conducted 10-min focal observations on each enclosure during each day of observations. We collected data on all skinks within an enclosure during each focal observation. Observations started between 0800 and 1030 hours, once >50% of all skinks had emerged from their overnight shelters. Daily observations concluded after either having recorded observations for all enclosures or once the skinks' active phase had ended (~1400 hours, but earlier on hot days >30°C) and >50% of all skinks had remained inside shelters for over 10 min. Observations were conducted from at least 3 m from enclosures to prevent avoidance and escape behaviours in skinks. To avoid temporal effects, the order in which enclosures were observed each day was randomised. During the 10-min focal observations, we monitored and timed skinks performing the following behaviours: (1) pair basking – basking within one body length of another skink on the same shelter; (2) pair sheltering – within the same shelter as another skink; (3) exploring – moving on floor or wall of enclosure; (4) tongue flicks; (5) bites; and (6) chases. A minimum of nine focal observations were conducted on each enclosure during each experiment. We used the average of these, that is, the average frequency and duration of each behaviour per 10-min observation period for each individual, in analyses.

Each morning of behavioural observations, we noted the overnight sheltering position and partners (if applicable) of the skinks in each enclosure. This was done before skinks emerged from their shelters, typically between 0730 and 0830 hours, again depending on weather conditions.

Blood-sampling procedure

We obtained blood by making a small incision in the *sinus angularis*, through the angle of the open jaw. We collected 100–200 µL of blood using heparinised capillary tubes; the blood was kept on ice for no longer than 5 h until centrifuged (3 min at 10 000 rpm). Isolated plasma was then frozen at −20°C until analysis using radioimmunoassay.

We collected blood from skinks on two occasions during each experiment, first at Day 3 then again at Day 17. These days were chosen to provide an insight into short-term and longer-term stress responses to the experimental manipulations. For Experiment 1, weather conditions, and hence skinks, on both Day 3 and Day 17 were cold (maximum temperatures of 21.6°C and 23.5°C) and limited our ability to obtain blood due to vasoconstriction. Consequently, after capture we warmed skinks to normal basking temperature in cloth bags on hot-water bottles for an average of 5 ± 1 min before blood sampling, bringing skinks to a normal basking temperature to facilitate blood collection. All skinks were sampled, on average, within 12 ± 2 min of capture, which is well below the 1 h necessary to induce a significant adrenocortical response to handling stress in a related species, *Egernia whitii* (Jones and Bell 2004). For Experiment 2, weather conditions were hot (maximum temperatures of 39.1°C and 41.9°C) so sampling commenced at ~0800 hours, and blood was collected, on average, 2 ± 1 min after capture. Blood-sampling protocol (time of sampling, order of sampling and handling time) was kept consistent within each experiment to minimise variation in basal corticosterone levels between samples (Jones and Bell 2004). Although there was a difference in post-capture sampling time between Experiments 1 and 2, our aim was to compare corticosterone levels within each experiment and not between experiments (although absolute levels of corticosterone were similar between experiments, suggesting that differences in the post-capture sampling time had minimal effect in this species).

Radioimmunoassay procedure

We measured plasma corticosterone using commercially available radioimmunoassay kits (Corticosterone 3H kits, MP Biomedicals, Solon, Ohio). We extracted 30 µL of plasma (containing 2000 CPM of tritiated corticosterone for estimating extraction efficiency) in 8 mL of diethyl ether. Extracted samples were reconstituted in 400 µL steroid buffer before assay. We followed the assay protocols outlined with each kit to measure plasma corticosterone. We calculated final corticosterone concentrations from standard curves and corrected for individual sample extraction recovery, plasma volume and addition of tritiated steroid. Average extraction efficiency for corticosterone over the four assays was 76.2% ± 1.05, and the minimum assay detection for corticosterone averaged 2.75 pg per tube. The inter- and intraassay coefficient of variation was 7.4% and 11.2%, respectively.

Statistical analyses

We tested for effects of treatment, sex and their interaction on the log-transformed values per 10-min focal observation for each of the behaviours (averaged per individual over the entire experimental period) using General Linear Mixed Models. We included 'enclosure number' as a random effect in models to account for non-independence of the behaviour of individuals within enclosures. To analyse differences in corticosterone, we used repeated-measures ANOVA, with time of sampling (early or late in the experiment) as the repeated-measure (within-subject effect), treatment and sex as between-subject effects and skink ID as the subject. We also tested for two-way interactions between

time of sampling, treatment and sex. As we were interested in relating changes in corticosterone over the experimental period with changes in behaviour, we also calculated mean values of each behaviour early and late in the experimental period. These were calculated as the mean of values from focal observations conducted over the five days closest to the day of blood sampling (Days 1–5 and 15–20 for Experiment 1 and Days 1–5 and 12–16 for Experiment 2). These data were analysed in the same way as the corticosterone data. All models were run using PROC MIXED, SAS ver. 9.1 and included enclosure ID as a random effect to account for non-independence of data for skinks within the same enclosure. Where a significant interaction was detected, we used one-way ANOVAs to determine significant differences for each factor separately.

To compare the frequency at which skinks sheltered together in the two treatments, we first calculated the frequency of pair sheltering expected by chance in each treatment (Table 1). We then calculated the ratio of the observed to expected frequency to enable comparison between treatments (Table 1). A ratio of 1.0 indicates that skinks sheltered as pairs at the same frequency expected by chance. We tested for an effect of treatment and sex on pair sheltering using both observed frequencies and the ratio of observed to expected frequencies (i.e. controlling for frequencies expected by chance).

Results

Behavioural responses to manipulation of population density

There were significant differences in the time that skinks spent pair basking and the frequency of chases between low- and high-population-density treatments. Skinks in low-density enclosures spent significantly more time pair basking than skinks in high-density enclosures ($F_{1,27} = 6.92$, $P = 0.01$). Chasing behaviour also differed, with skinks at high-population densities chasing or being chased significantly more often than skinks at low densities ($F_{1,27} = 4.93$, $P = 0.04$). There was a significant difference between the sexes (but not between treatments) in the time skinks spent exploring their enclosure, with males spending more time exploring than females ($F_{1,27} = 4.59$, $P = 0.04$). However, there were no significant effects of treatment or sex on the time skinks spent sheltering as pairs or the frequency of bites and tongue flicks.

Skinks sheltered as pairs significantly more often in the later part of the experiment than in the beginning ($F_{1,67} = 13.31$, $P = 0.0005$). There was a significant interaction between time and treatment for pair basking ($F_{1,67} = 7.87$, $P = 0.007$). Skinks

basked in pairs significantly more often towards the end of the experiment in the low-skink-density treatment ($F_{1,20} = 11.85$, $P = 0.001$) but not the high-density treatment ($F_{1,49} = 2.23$, $P = 0.14$). There was also a weak trend for a decrease in the frequency of bites over time in males (sex × time interaction: $F_{1,67} = 2.97$, $P = 0.089$).

Skinks sheltered overnight with other skinks more often than expected by chance in low-population-density enclosures, whereas in high-population-density enclosures they sheltered together at frequencies similar to that expected by chance (Table 1). After controlling for frequencies of sheltering together expected by chance, there was no significant difference in the frequency of sheltering overnight together versus alone between skinks in low- and high-population-density treatments for both males ($F_{1,17} = 2.79$, $P = 0.11$) and females ($F_{1,17} = 3.44$, $P = 0.08$).

Physiological responses to manipulation of population density

There were no significant overall differences in corticosterone levels between the sexes ($F_{1,46} = 0.02$, $P = 0.9$) or between treatments ($F_{1,46} = 3.35$, $P = 0.07$). However, there was a significant effect of time on corticosterone levels, with skinks having greater levels of circulating corticosterone early in the experiment compared with late in the experiment ($F_{1,46} = 5.07$, $P = 0.03$) and the interaction between sex and treatment approached significance ($F_{1,46} = 3.76$, $P = 0.06$). One-way ANOVAs revealed that males in high-density enclosures had significantly higher levels of plasma corticosterone than did males at low densities ($F_{1,22} = 5.58$, $P = 0.03$), but that there was no significant difference between females in high- and low-density treatments ($F_{1,19} = 0.01$, $P = 0.94$) (Fig. 1).

Behavioural responses to manipulation of shelter density

There were no significant differences in the duration or frequency of any measured behaviour between either shelter treatments or between the sexes. However, there were significant interactions between sex and treatment for both tongue flicks ($F_{1,19} = 13.83$, $P < 0.01$) and enclosure exploration ($F_{1,19} = 11.45$, $P < 0.01$). One-way ANOVAs revealed that males explored their enclosures significantly more often in low- than in high-shelter-density treatments ($F_{1,18} = 12.83$, $P = 0.002$) (Fig. 2). However, there was no significant difference for females in the frequency of exploring behaviours between treatments ($F_{1,19} = 0.01$, $P = 0.93$) (Fig. 2a). Males tongue flicked significantly more than females in low-shelter-density enclosures ($F_{1,19} = 24.35$, $P < 0.001$) (Fig. 3) but there was no difference between the sexes in frequency of tongue

Table 1.   Observed and expected frequency of sheltering overnight with other skinks

Expected frequency is the average frequency of sheltering overnight with other skinks expected by chance, with the ratio of observed to expected frequency calculated to allow comparisons between treatments. A ratio of 1.0 indicates levels of pair sheltering are as expected by chance

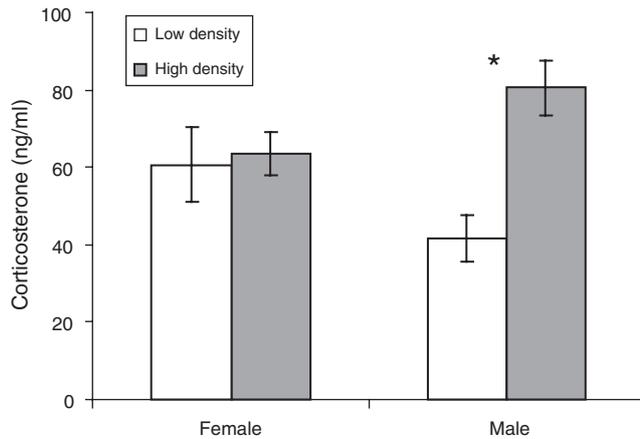| Experiment	Density	Sex	Observed pair sheltering frequency	Expected pair sheltering frequency	Ratio (observed to expected)
Population-density manipulation	Low	Both	0.67	0.5	1.34
	High	Male	0.89	0.86	1.04
		Female	0.87	0.86	1.01
Shelter-density manipulation	Low	Both	1.83	0.5	3.36
	High	Both	0.48	0.25	1.92

Fig. 1. Mean (±s.e.) plasma corticosterone levels of male and female skinks in low- and high-population-density treatments. Significant differences (*P* < 0.05) are marked with an asterisk.
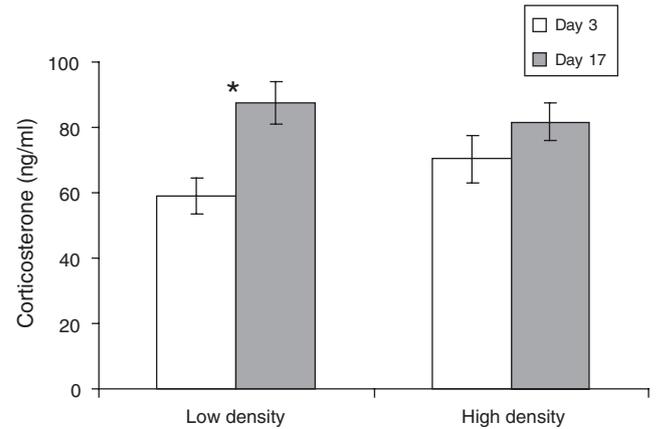

Fig. 3. Mean (±s.e.) plasma corticosterone levels of skinks in low- and high-shelter-density treatments early (Day 3) and late (Day 17) in the experiments. Significant differences (*P* < 0.05) are marked with an asterisk.
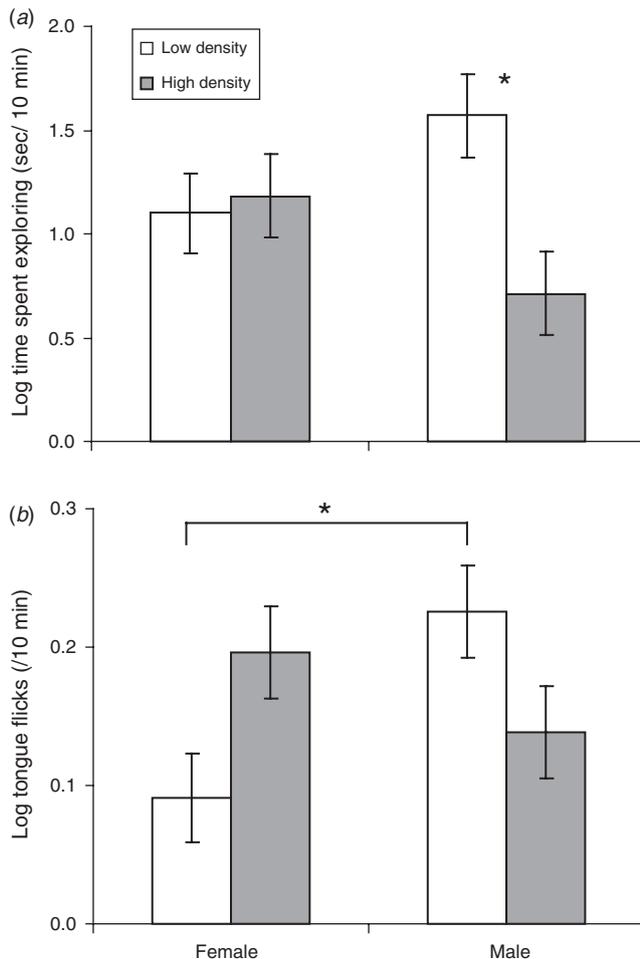

Fig. 2. Log-transformed mean (±s.e.) (*a*) duration of enclosure exploring behaviours and (*b*) frequency of tongue flicks performed by males and females in high- and low-shelter-density treatments. Significant differences (*P* < 0.05) are marked with an asterisk.

flicks in high-shelter-density enclosures ($F_{1,18}$ = 0.91, *P* = 0.354) (Fig. 2*b*). There was no significant difference in the frequency of any behaviour between early and late stages in the experimental period (*P* > 0.2 in all cases). Hence, behaviours within each treatment remained consistent over time.

Skinks sheltered overnight in pairs at both low- and high-shelter density more often than expected by chance. Furthermore, skinks in low-shelter-density enclosures sheltered overnight together significantly more often than skinks in high-shelter-density enclosures ($F_{1,18}$ = 18.23, *P* < 0.01) (Table 1).

Physiological responses to manipulation of shelter density

There were no significant overall differences between treatments ($F_{1,34}$ = 0.06, *P* = 0.81) or between the sexes ($F_{1,34}$ = 0.17, *P* = 0.68) in circulating levels of corticosterone. However, there was a significant effect of sampling time (with an increase in corticosterone over time; $F_{1,34}$ = 6.16, *P* = 0.02) and the interaction between time and treatment approached significance ($F_{1,34}$ = 3.94, *P* = 0.06). One-way ANOVAs revealed that skinks in low-shelter-density enclosures had greater levels of circulating corticosterone at the end of the experiment than at the start ($F_{1,14}$ = 10.09, *P* < 0.01) (Fig. 3) whereas skinks in high-shelter-density enclosures showed no significant differences over time ($F_{1,14}$ = 0.21, *P* = 0.65) (Fig. 3).

Discussion

The results of our experiments suggest that both population density and shelter density influence the social behaviours of the tree skink. Higher population density is expected to increase the frequency of interactions, potentially leading to either increased affiliative behaviours or increased agonistic behaviours. However, at very high population densities agonistic interactions can result in social disruption and inhibit social group formation (Judge and DeWaal 1997). This could explain why skinks at high population densities spent less time basking as pairs during the day. The absence of any possible intrasexual conflict in low-density enclosures is likely to promote increased pair basking

and lower frequency of chases observed. Consistent with this explanation, skinks at high densities exhibited more frequent agonistic behaviours, specifically chasing. Most agonistic interactions occurred between males, although agonistic interactions were also observed between males and females and between females. High intrasexual aggression is consistent with results from the wild, in which tree skink social groups typically contain only one adult male (Bonnett 1999). Other *Egernia* species that form social groups containing one or few males have also been shown to be aggressive towards conspecifics (O'Connor and Shine 2004; While *et al.* 2009*a*). With increased population density, elevated intraspecific aggression can greatly influence an individual's ability to acquire territory and mates, and disperse and form social groups (Sinn *et al.* 2008; While *et al.* 2009*b*). We sometimes observed three or four skinks within two metres of each other in the field, but could not determine their age and sex. Males may therefore rarely be found in as close proximity in the wild as they were in our high-density enclosures, thereby elevating density effects on aggression. Nevertheless, aggressive behaviours were relatively rare even in high-density enclosures, particularly in comparison to observations of other *Egernia* species (O'Connor and Shine 2004).

Increased aggressive behaviours at high densities were associated with increased corticosterone in males in high- compared with low-population-density enclosures. Prolonged aggression and social instability have fitness costs to individuals (Rubenstein 2007), with energy loss and an increased risk of injury as a result of bites or chases. One way that animals can reduce these costs is to form dominance hierarchies (Van Meter *et al.* 2009). Once dominance status is established, the frequency of agonistic behaviours and aggression often decreases, occurring typically only during rank challenges (Archer 1988). This may explain why corticosterone levels decreased over time in Experiment 1, with skinks settling into dominant and subordinate positions. This view is reinforced by the increase in pair sheltering and trend for a decrease in bites inflicted by males within the high-density treatment.

Females at high- and low-skink densities did not differ in corticosterone levels despite differences in levels of aggression between treatments. This lack of difference in female corticosterone could potentially be explained by at least two mechanisms. First, while chases or bites (aggressive behaviours) did not differ significantly between the sexes, the initiation, maintenance and perception of social aggression is generally more prevalent in males (Archer 1988). Hence, if male tree skinks perceive more aggression relative to females, this may result in different corticosterone levels in males exposed to the high- or low-population-density treatments but not in females. The second possible explanation is that the stress responses of some female vertebrates, including reptiles, is attenuated during reproduction, causing a reduction in the corticosterone stress response relative to males (Moore and Jessop 2003).

Although daytime pair behaviours did not differ between shelter density treatments, as predicted, skinks in low-shelter-density enclosures sheltered overnight in pairs significantly more often than those in high-shelter-density enclosures, even after controlling for frequencies of pair sheltering expected by chance. A shortage of available shelters is often proposed to explain aggregation and the evolution of social groups in reptiles (Chapple 2003; Langkilde and Shine 2004) and other social vertebrates (Hatchwell and Komdeur 2000; Finstad *et al.* 2007) and has been specifically proposed to represent the first step towards social organisation within this genus (Chapple 2003). The frequency of pair sheltering in the low-shelter-density treatment was substantially greater than expected by chance, suggesting that shelter density could influence the social organisation of the tree skink, by promoting aggregative behaviours and increasing social interactions.

Even with an excess of available shelters, skinks in high-shelter-density enclosures still frequently sheltered as pairs, suggesting that there may be other factors promoting pair or group formation, such as predation pressure (e.g. Lanham and Bull 2004), or thermoregulatory and other physiological benefits, which promote social groupings and aggregations in geckos and other reptiles (Lancaster *et al*. 2006; Reiserer *et al.* 2008). However, these explanations have primarily been proposed to explain aggregation and may be less likely in species with more complex forms of social organisation, such as *Egernia*, in which pairing may be more likely to reflect attempts to form pair bonds.

In contrast to the population-density experiment, skinks in low-shelter-density enclosures exhibited higher levels of corticosterone later in the experiment. Increasing plasma corticosterone levels over time in low-shelter-density enclosures is not intuitively explained by social stressors, as absolute levels of aggression were low and did not differ between treatments. Males in the low-shelter-density treatment also showed the highest frequencies of both tongue flicks and exploration behaviour. Increases in locomotory and exploratory behaviour in other lizard species have been attributed, in part, to weather conditions or increased levels of corticosterone (Belliure and Clobert 2004). For three days before blood sampling on Day 17 of Experiment 2, maximum temperatures exceeded 35°C (with ground temperatures presumably hotter), which may have caused a stress response in skinks. Hence, high temperatures may well explain the greater corticosterone levels and resultant increases in stress-related behaviours such as exploratory behaviours exhibited by skinks at low densities compared with the high-shelter-density treatment. Although shelters were arranged to minimise variation in thermal properties, with each facing the same direction and receiving equal sunlight, an increase in shelter number (i.e. high shelter density) may have provided increased microclimate variation for skinks to avoid physiological stress. It is possible that temperature-related stress responses may have masked or altered any potential effect of shelter density treatment on corticosterone levels.

In summary, our results suggest that the independent effects of population density and shelter density can elicit changes in specific behaviours and corticosterone responses in tree lizards. Although our experiments were designed to examine short-term aggregations and social behaviour rather than long-term stable social organisation, our results suggest that ecological factors influence aggregative behaviour in this species, which exhibits habitat-dependent differences in social aggregations. However, common garden experiments using populations sourced from different habitats are needed to determine whether the differing tendencies to aggregate reflect a heritable genetic difference or a plastic response to ecological conditions. More broadly, our results are consistent with the view that affiliative behaviours as

a result of habitat saturation may have represented the first step towards the evolution of social organisation within this genus. Finally, our study contributes to our understanding of the extreme variation in levels of aggregation and social complexity found in *Egernia* lizards, which can provide important insights into the evolution of vertebrate sociality.

Acknowledgements

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