



Effects of chronic corticosterone increases on the maternal behaviour of the prairie skink, *Plestiodon septentrionalis*

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Maternal care behaviour is rare in reptiles and the hormonal control of this behaviour is less well known than in other vertebrates. The steroid hormone, corticosterone, suppresses maternal behaviour in avian species. We investigate whether corticosterone similarly affects maternal behaviour of a lizard. We artificially elevated corticosterone in female prairie lizards, *Plestiodon septentrionalis*, during egg brooding and assessed effects on maternal behaviour (versus females receiving a vehicle control). The application of exogenous corticosterone significantly decreased the amount of time that females spent in contact with their eggs. These results suggest that, as in birds, corticosterone acts to reduce maternal behaviours in reptiles. This provides important insight into the hormonal control of, and effects of stress on, parental care in reptiles.

Key words: Brooding, stress, eggs, hormone, lizard, parental care, reptile

INTRODUCTION

Parental behaviour in reptiles appears to be relatively rare but does exist. Parental care is widespread in crocodilians (Somma, 2003) and, although there have been relatively few studies of parental care in squamates, there is evidence of nest defence in skinks (Huang & Pike, 2011a,b), shivering thermogenesis in pythons (Aubret et al., 2005) and the suggestion that skinks and pythons actively maintain appropriate moisture levels within their nest (Somma & Fawcett, 1989; Lourdais et al., 2007). Females of some viperid (Hoss & Clark, 2014; Greene et al., 2002) and scincid species (Langkilde et al., 2007; O'Connor & Shine 2004) also remain associated with their young and provide them care and protection. While these studies have advanced our knowledge of reptilian parental behaviours, there are few studies addressing the hormonal control of this behaviour (but see Hoss et al., 2014; Lind et al., 2017).

There has been a great deal of research on the hormonal control of parental behaviour in birds, where parental care is nearly universal. It is generally accepted that prolactin (PRL) induces parental behaviour in birds (Buntin et al., 1991) and recent studies have revealed that PRL can interact with the stress hormone corticosterone (CORT), suggesting a possible role of CORT in parental behaviour. Specifically, increased CORT can cause a decrease in PRL, which leads to decreased parental effort and in extreme cases, nest abandonment (Chastel & Lormee, 2005; Chastel et al., 2005; Groscolas et al.,

2008; Angelier et al., 2009). Reptiles are closely related to birds and so CORT may similarly affect parental care in reptiles.

We test the effect of CORT on maternal care behaviour in the Northern Prairie Skink, *Plestiodon septentrionalis*, a small lizard (70 mm SVL) that occurs from Kansas to Canada. *Plestiodon septentrionalis* and closely related species are known to exhibit parental care in the form of egg brooding (Breckenridge, 1943; Noble & Mason, 1993; Somma, 2003). During egg brooding, the female remains with the eggs until hatching, exhibiting near constant nest attendance (Breckenridge, 1943). During this time, the female may reposition eggs within the nest, retrieve displaced eggs, or coil her body around the eggs (Table 1). The female may even consume eggs that become infected with fungus in order to prevent the infection spreading to the other eggs (Somma, 1989). We experimentally elevated plasma CORT of female *P. septentrionalis* twice daily during brooding and quantified effects on maternal brooding behaviour.

MATERIALS AND METHODS

Study species

Eight female and eight male skinks were captured from Pawnee and Douglas Counties, Nebraska in April and May 2012. Reproductive maturity was determined by orange mating colouration on the neck in males and SVL above 60 mm in females (Ballinger et al., 2010). All skinks were returned to the laboratory at the University of Nebraska

Table 1. Descriptions of maternal brooding behaviours recorded in this study (from Somma & Fawcett, 1989)

Name of behaviour	Description
<i>Positional Behaviours</i>	
Contact	Female positions herself in contact with the eggs.
Coiling	Female tightly coils around eggs.
No Contact	Female is under the shelter but is not in contact with the eggs.
Not Present	Female is absent from the shelter.
<i>Active Behaviours</i>	
Biting	Female bites an egg.
Digging	Female excavates the nest or area around it using her forelimbs.
Nudging	Female nudges an egg but does not change its position.
Pushing	Female changes the position of an egg without overturning it.
Rolling	Female changes the position of an egg by overturning it.

at Omaha. Each female was temporarily housed with a single male for mating. Once females were gravid, as indicated by abdominal swelling, males were removed from the enclosure. Enclosures consisted of 37.85 litre aquariums filled with 2,000 grams of commercial topsoil mixed with 500 grams of sand. Each enclosure contained a translucent red acrylic plate (15 x 15.5 cm, L x W) for shelter and for females to nest beneath. These plates allowed us to view females through the shelters using night vision cameras without disturbing them. Room lighting was on from 0600h to 2000h. Ultraviolet lighting (R-Zilla UVA/UVB 48”) in reflective hoods was hung 1.5 metres above enclosures and 50-watt heat lamps were positioned over one side of each enclosure to allow the lizard to thermoregulate. Lizards were fed to satiation daily and water was available ad libitum.

Experimental design

Females were randomly assigned to one of two treatments, CORT application or vehicle control, which they received each day at 0800h and 1400h. CORT application began the day after oviposition. The CORT application treatment consisted of 45ug CORT dissolved in 4.5ul of sesame oil and the control treatment consisted of 4.5ul of sesame oil (n=4 for each treatment group). Treatments were applied to the lizard’s backs using a pipette. Females were not handled during this process. If females were on the nest, the shelters were lifted slightly to facilitate application. The lipophilic nature of lizard skin means that the oil or oil/hormone mixture is quickly absorbed (Belluore & Clobert, 2004). This treatment method has been previously used to temporarily increase plasma CORT levels in reptiles (Knapp & Moore, 1997; Cote et al., 2006; Trompeter & Langkilde, 2011). A pilot study revealed that this CORT application regime resulted in elevated plasma CORT concentrations for *P. septentrionalis* at 6 hrs post application (baseline mean = 39.1 ng/ml, post CORT-application mean = 67.2 ng/ml; repeated measures ANOVA: F1,9 = 4.91, p=0.054;

A. Anton & T. Langkilde, unpublished data). Soil samples were carefully taken from the edge of each nest at 0830h, after females had nested. This rarely scared females off the nest. These were analysed for moisture content and water was added to the nest as necessary to maintain these at 20% moisture. Video trials began at 0930h the day after the females had laid their eggs and ended at 1330h each day. Two camcorders simultaneously recorded behaviour of two randomly selected females for one hour, and were then moved to another pair of females. Each female was video recorded for one hour per day and the order in which the females were recorded was rotated each day. Video trials continued daily until the female’s eggs were determined either unviable, as indicated by a shrivelled or mouldy appearance, or had hatched. The number of eggs that successfully hatched was recorded for each female.

Analysis

Video recordings were analysed using Windows Media Player 12 (Microsoft, Redmond, WA). Videos were scored blind to treatment. Two types of behaviours were quantified: positional and active (as per Somma & Fawcett, 1989). Positional behaviours describe the female presence and position in relation to the eggs within the nest and have been suggested to alter moisture loss and gain of eggs (Somma & Fawcett, 1989) (Table 1). Active behaviours are those in which an individual physically interacted with the nest or eggs (Table 2). Both types of behaviour were recorded using presence-absence sampling at 30-second intervals. The total observations of each behaviour were weighted for the number of observations made and used in analyses. Data are presented in figures as percentage of observations a female exhibited for each behaviour. Data were analysed using separate Mann-Whitney U tests in Minitab 17 (Minitab Inc., State College, PA) with the weighted occurrence of each behaviour as the dependent variable and treatment as the factor.

RESULTS

Positional Behaviours

CORT-treated females tended to spend less time present at the nest and, when on the nest, were less likely to be in contact with the with the eggs and spent less time coiled around the eggs than did females in the control group (Table 2, Figs. 1 and 2). For all other positional behaviours, no significant effect of CORT treatment was found (Table 2).

Active Behaviours

No significant effects of CORT treatment were found between control and experimental groups for any of the five active behaviours (Table 2).

DISCUSSION

We found effects of application of CORT on maternal-care behaviour; females treated with CORT after oviposition tended to spend more time away from the nest. When CORT-treated females were at the nest, they spent

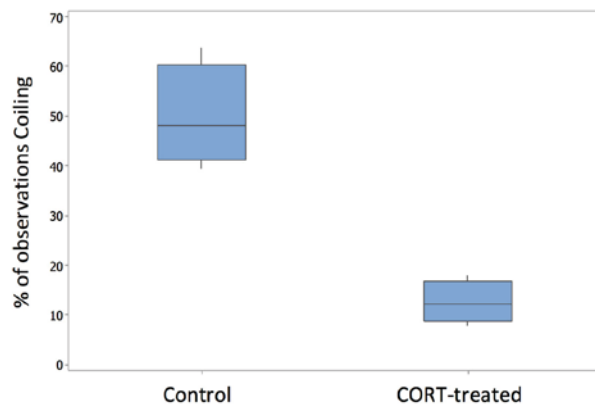


Figure 1. The percentage of observations in which female *P. septentrionalis* in the CORT-application and control treatment were coiled around their eggs ($n = 4$ per treatment). The box encompasses the 1st to 3rd quartile of the samples. The line within the box indicates the median value. The top and bottom of each vertical line represents the maximum and minimum values.

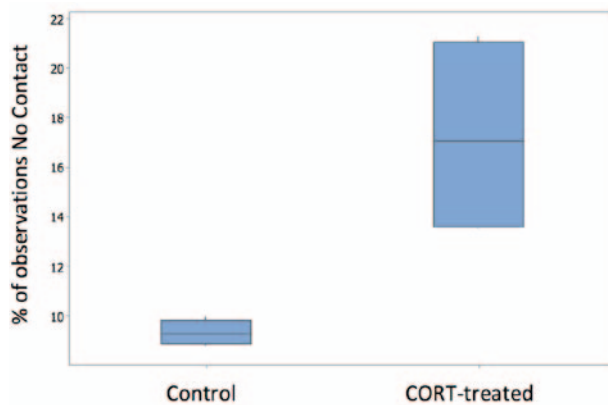


Figure 2. Percentage of observations in which female *P. septentrionalis* in the CORT-application and control treatment were within the nest but not in contact with their eggs ($n = 4$ per treatment). The box encompasses the 1st to 3rd quartile of the samples. The line within the box indicates the median value. The top and bottom of each vertical line represents the maximum and minimum values.

Table 2. The results of Mann-Whitney tests for the effect of treatment (application of corticosterone versus vehicle control) on behaviour of *P. septentrionalis*. $n = 4$ per treatment. Significant results are indicated by bold text.

Name of behaviour	Z	P
<i>Positional Behaviours</i>		
Contact	0.13	0.19
Coiling	-0.36	0.03
No Contact	0.08	0.03
Not Present	0.16	0.06
<i>Active Behaviours</i>		
Biting	>0.001	0.67
Digging	0.01	0.66
Nudging	-0.01	0.67
Pushing	0.01	0.89
Rolling	0.01	0.31

significantly less time in any contact with their eggs and less time coiled around the eggs. If the primary function of maternal care in *P. septentrionalis* is to maintain moisture levels as has been previously suggested (Somma & Fawcett, 1989), then less time spent in the nest in contact with the eggs could lead to detrimental conditions and lower hatching success. In our experiment, despite differences in female behaviour, hatching success was low in both the treatment and control groups (only two control females had at least one egg hatch and no eggs from CORT-treated females hatched), possibly due to the soil composition of our artificial nests, and so we were unable to determine the consequences of this change in behaviour for offspring. It is also possible that the nesting substrate and low viability of eggs could have impacted maternal behaviour. Future research should test for additional CORT-associated changes in maternal-care behaviour and for implications of these changes for the females (e.g., effects on body condition) and their offspring (e.g., survival, body condition).

Our results suggest that increases in CORT may decrease parental behaviour in reptiles, as it does in birds (Groscolas et al., 2008; Angelier et al., 2009). CORT has been implicated in the natural cessation of maternal care in cottonmouth snakes (Hoss et al., 2014). This effect may also be mediated by an interaction between CORT and the hormone prolactin, which is primarily responsible for the induction and maintenance of parental behaviour in birds (Buntin et al., 1991), but the mechanism is currently unknown. In species of reptile that exhibit parental care, elevations in CORT induced by anthropogenic activities such as urbanisation (French et al., 2008), pollution (Wikelski, 2001), tourism (Romero & Wikelski, 2002; French et al., 2010) or the introduction of non-native species (Graham et al., 2012) may thus negatively affect parental behaviour and therefore reproductive success.

Even with a small sample size, significant effects of CORT on maternal care behaviour of *P. septentrionalis* were detected, indicating that future studies on this phenomenon would be useful. Further research on the relationship between CORT and maternal care in this and other reptile species would shed important light onto the hormonal controls of this behaviour, and possible implications of increased stress.

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REFERENCES

- Angelier, F., Clement-Chastel, C., Welcker, J. & Chastel, O. (2009). How does corticosterone affect parental behavior and reproductive success? A study of prolactin in black-legged kittiwakes. *Functional Ecology* 23, 784-793.
- Aubret, F., Bonnet, X., Shine, R. & Maumelat, S. (2005). Energy expenditure for parental care may be trivial for brooding pythons, *Python regius*. *Animal Behaviour* 69, 1043-1053.
- Ballinger, R.E., Lynch, J.D. & Smith, G.R. (2010). *Amphibians and Reptiles of Nebraska*. Oro Valley, Arizona, Rusty Lizard Press.
- Belliure, J. & Clobert, J. (2004). Behavioral sensitivity to corticosterone in juveniles of the wall lizard *Podacris muralis*. *Physiological Behavior* 81, 121-127.
- Breckenridge, W.J. (1943). The life history of the black-banded skink *Eumeces septentrionalis septentrionalis*. *American Midland Naturalist* 29, 591-606.
- Buntin J., Becker, G. & Ruzyski, E. (1991). Facilitation of parental behavior in ring doves by systemic or intracranial injections of prolactin. *Hormones and Behavior* 25, 424-444.
- Chastel O., Lacroix, A., Weimerskirch, H. & Gabrielsen, G.W. (2005). Modulation of prolactin but not corticosterone responses to stress in relation to parental effort in a long-lived bird. *Hormones and Behavior* 47, 459-466.
- Chastel, O. & Lormee, H. (2005). Patterns of prolactin secretion in relation to incubation failure in a tropical seabird, the red-footed booby. *Condor* 104, 873-876.
- Cote, J., Clobert, J., Meylan, S. & Fitze, P.S. (2006). Experimental enhancement of corticosterone levels positively affects subsequent male survival. *Hormones and Behavior* 49, 320-327.
- French, S., Fokidis, H. & Moore, M. (2008). Variation in stress and innate immunity in the tree lizard (*Urosaurus ornatus*) across an urban-rural gradient. *Journal of Comparative Physiology B* 178, 997-1005.
- French, S., Denardo, D.F., Greives, T.J., Strand, C.R. & Demas, G.E. (2010). Human disturbance alters endocrine and immune responses in the Galapagos marine iguana (*Amblyrhynchus cristatus*). *Hormones and Behavior* 58, 792-799.
- Graham, S.P., Freidenfelds, N.A., McCormick, G.L. & Langkilde, T. (2012). The impacts of invaders: basal and acute stress glucocorticoid profiles and immune function in native lizards threatened by invasive ants. *General and Comparative Endocrinology* 176, 400-408.
- Greene, H. W., May, P. G., Hardy Sr, D. L., Scituro, J. M. & Farrell, T. M. (2002). Parental behavior by vipers. In G. W. Schuett, M. Höggren, M. E. Douglas, & H. W. Greene (Eds.), *Biology of the Vipers* (pp. 179-205). Eagle Mountain Utah: Eagle Mountain Publishing.
- Groscolas, R., Lacroix, A. & Robin, J. (2008). Spontaneous egg or chick abandonment in energy-depleted king penguins: A role for corticosterone and prolactin? *Hormones and Behavior* 53,1: 51-60.
- Hoss, S. K., Garcia, M. J., Earley, R. L. & Clark, R. W. (2014). Fine-scale hormonal patterns associated with birth and maternal care in the cottonmouth (*Agkistrodon piscivorus*), a North American pitviper snake. *General and Comparative Endocrinology* 208, 85-93.
- Hoss, S. K. & Clark, R. W. (2014). Mother cottonmouths (*Agkistrodon piscivorus*) alter their antipredator behavior in the presence of neonates. *Ethology* 120, 933-941.
- Huang, W.S. & Pike, D.A. (2011a). Does maternal care evolve through egg recognition or directed territoriality? *Journal of Evolutionary Biology* 24, 1984-1991.
- Huang, W.S. & Pike, D.A. (2011b). Determinant of homing in nest-guarding females: balancing risks while travelling through unfamiliar landscapes. *Animal Behaviour* 882, 263-270.
- Knapp, R. & Moore, M.C. (1997). A non-invasive method for sustained elevation of hormone levels in reptiles. *Herpetological Review* 28, 33-35.
- Langkilde, T., O'Connor, D. & Shine, R. (2007). The benefits of parental care: do juvenile lizards obtain better-quality habitat by remaining with their parents? *Austral Ecology* 32, 950-954.
- Lind, C. M., Birky, N. K., Porth, A. M. & Farrell, T. M. (2017). Vasotocin receptor blockade disrupts maternal care of offspring in a viviparous snake, *Sistrurus miliarius*. *Biology Open* bio-022616.
- Lourdais, O., Hoffman Ty, C.M. & DeNardo, D.F. (2007). Maternal brooding in the children's python (*Antaresia childreni*) promotes egg water balance. *Journal of Comparative Physiology B* 177, 569-577.
- Noble, G.K. & Mason, E.R. (1933). Experiments on the brooding habits of *Eumeces* and *Ophisaurus*. *American Museum novitates* 619, 1-29.
- O'Connor, D. & Shine, R. (2003). Lizards in 'nuclear families': a novel reptilian social system in *Egernia saxatilis* (Scincidae). *Molecular Ecology* 12, 743-752.
- Romero, L.M. & Wikelski, M. (2002). Exposure to tourism reduces stress-induced corticosterone levels in Galapagos marine iguanas. *Biological Conservation* 108, 371-374.
- Somma, L.A. (1989). Oophagous behavior in brooding prairie skinks, *Eumeces septentrionalis*. *Herpetological Review* 20, 3-4.
- Somma, L.A. (2003). *Parental behavior in lepidosaurs and testudinian reptiles*. Krieger Publishing Company: Malabar, Florida.
- Somma, L.A. & Fawcett, J.D. (1989). Brooding behaviour of the Prairie Skink, *Eumeces septentrionalis*, and its relationship to the hydric environment of the nest. *Zoological Journal of the Linnean Society* 95, 245-256.
- Trompeter, W.P. & Langkilde, T. (2011). Invader Danger: lizards faced with novel predators exhibit an altered behavioral response to stress. *Hormones and Behavior* 60, 152-158.
- Wikelski, M. (2001). Marine iguanas oiled in the Galapagos. *Science* 292, 437-438.

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