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Effects of parasitic infection and radio-transmitters on condition, hematological characteristics and corticosterone concentrations in Texas ratsnakes

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Abstract

Parasites are a health concern of many wildlife species, although their effect on reptiles is poorly studied. Here we examine effects of hemogregarine and external parasites on Texas ratsnake *Elaphe obsoleta* white blood cell (WBC) concentrations, WBC differentials, heterophil/lymphocyte ratios, packed cell volume (PCV), corticosterone concentrations and body condition. We found little evidence that either ectoparasites or hemogregarines affected the health of Texas ratsnakes. We found a seasonal increase in corticosterone, consistent with glucocorticoids priming the snakes to deal with higher predation risks later in the season. We also examined whether the potential stress of carrying a surgically implanted radio-transmitter compounds any parasitic health effects. We found no effect of transmitters on most aspects of health. We did find equivocal evidence that WBC concentrations were higher in snakes with transmitters and that PCV decreased following transmitter implantation. Although our results generally support the view that data collected using radio-telemetry are unlikely to be biased due to adverse health effects of transmitters, the growing importance of telemetry for studies of snakes and other taxa argues for further research on this topic. When possible, researchers should design telemetry studies in such a way that assessing potential heath effects of transmitters is one of the objectives.

Introduction

Parasites affect host life history (Michalakis & Hochberg, 1994), behavior (Moore, 1984) and evolution (Buckling & Rainey, 2002). Although parasites are widespread, much of the work examining host–parasite interactions on wild animals has focused on birds, particularly following the proposal by Hamilton & Zuk (1982) that the colorful plumage exhibited by many bird species evolved as a signal of health and parasite resistance. While evidence is steadily growing regarding health effects of parasites in birds, relatively little work has been done to address similar issues in reptiles (Jacobson, 2007) and the available evidence is contradictory. Some snake species exhibit decreased growth and body condition (Madsen, Ujvari & Olsson, 2005) or reduced immune response (Ujvari & Madsen, 2006) in response to parasites, whereas others exhibited no deleterious effects (Caudell, Whittier & Conover, 2002; Brown, Shilton & Shine, 2006). The relationship between parasites and stress in reptiles has also received recent attention, with environmental stressors increasing blood parasite infection in lizards (Oppliger *et al*., 1998; Amo, López & Martín, 2007).

Glucocorticoids are often used to measure health in wild animals. High plasma concentrations of glucocorticoids are correlated with low body mass (Dunlap & Wingfield, 1995; Moore *et al*., 2000), higher parasite load (Dunlap & Schall, 1995; Raouf *et al*., 2006), lower wound-healing rates (French, Matt & Moore, 2006), presence of a predator (Scheuerlein, Van't Hof & Gwinner, 2001; Cockrem & Silverin, 2002) and promote self-maintenance over reproduction (Wingfield & Romero, 2001). Perhaps counterintuitively, however, chronic stress can lower glucocorticoid concentrations (Rich & Romero, 2005; Cyr & Romero, 2007), a relationship observed in iguanas exposed to long-term anthropogenic disturbance (Romero & Wikelski, 2002). Similarly, blood parasites were correlated with lower corticosterone levels in juvenile iguanas (Hanley & Stamps, 2002). Thus, caution is required when predicting how corticosterone will change in response to stress caused by human disturbance and parasites.

Our objective was to test the hypothesis that parasites negatively affect health of Texas ratsnakes *Elaphe obsoleta*, including changes in hematological characteristics [i.e. anemia, increased leukocyte concentrations and altered white blood cell (WBC) differentials] and body condition. We also

investigated the relationship between parasitism and corticosterone concentrations. Finally, we took advantage of a concurrent study in which we implanted snakes with radio-transmitters, to test the prediction that transmitters would increase stress, reflected by altered corticosterone concentrations and increased parasitic infection.

Research involving snakes has increased substantially (Shine & Bonnet, 2000), due in part to the development of miniature, surgically implanted transmitters. Despite the widespread adoption of radio-telemetry in field studies of snakes, however, there is little information regarding potential health effects of transmitters. Weatherhead & Blouin-Demers (2004) found reduced growth and relative clutch mass in ratsnakes with radio-transmitters relative to snakes without, potentially resulting from infection from the surgery- or from researcher-induced stress from repeated disturbance (Weatherhead & Blouin-Demers, 2004). We test both these hypotheses using hematological values and corticosterone levels in Texas ratsnakes. We also determine whether transmitters exacerbate any health effects of parasites.

Materials and methods

We conducted this study from 2006 to 2007 at Fort Hood, Texas (30°10′N, 97°45′W). Snakes were caught opportunistically throughout the field season and measured (snout–vent length) and weighed. Snakes too small to be fitted with transmitters or captured outside our primary study areas were measured, bled and released. Snakes for which transmitters weighed $<3\%$ total body mass had transmitters surgically implanted and were released within several days at their capture locations. Transmitters (Model SI-2T, Holohil Systems Incorporated, Carp, ON, Canada) were implanted under sterile conditions by veterinarians using methods described by Reinert & Cundall (1982) as modified by Blouin-Demers & Weatherhead (2001). Snakes were relocated approximately every 48 h. We opportunistically recaptured snakes with transmitters at least once per year and collected an additional blood sample to assess changes in health after carrying a transmitter for a known time period.

We collected 0.1–0.4 mL of blood from the caudal vein using a syringe and 26 G needle. Blood sampling was completed within 5 min of capture for 20 of our 23 samples, and 12–15 min after capture for three samples. We included all samples in our analyses because corticosterone concentrations of samples collected 12, 15 and 15 min after capture were 4.5, 5.8 and 2.9, respectively, which is similar to the mean (6.3 ± 1.2) of samples collected within 5 min ($t = -0.07$, $P = 0.95$). Blood was immediately transferred to a heparinized tube and placed in a cooler. Within 24 h of collection we: (1) created a blood smear; (2) determined packed cell volume (PCV) (hematocrit); and (3) counted WBCs (eosinophils and heterophils, hereafter referred to as WBC concentrations). Blood smears were air dried, fixed in ethanol and stained with Giemsa stain. Blood plasma was frozen (−20 °C) until corticosterone assay.

Using a hand lens, we visually inspected all snakes for presence of external parasitic mites (family Trombiculidae, commonly known as chiggers, an ectoparasite of a variety of snake species; Brennan & Loomis, 1959), but did not estimate numbers of parasites. To quantify blood parasites from blood smears we used a microscope with $\times 100$ oil immersion objective and counted the number of hemogregarines per 5000 red blood cells. Hemogregarines are the most common blood parasites found in snakes (Telford, 1984). For this study, we were interested in both prevalence (the proportion of snakes infected) and intensity (number of parasites per sample) of hemogregarine infections.

Leukocyte counts were used to determine whether snakes were mounting an immune response against a bacterial, viral or parasitic infection. We counted eosinophil and heterophil cells using the Eosinophil Unopette staining system (Becton Dickinson, Franklin Lakes, NJ, USA) and a hemocytometer. We conducted three counts per sample and used the average of the counts for analysis. WBC concentrations (eosinophil and heterophil cells per μL) were determined using the formula provided by Strik, Alleman & Harr (2007).

To determine WBC differentials, we counted *c.* 100 leukocytes per blood smear using a microscope with $\times 100$ oil immersion objective. Cells were classified as heterophils, azurophils, monocytes, basophils, eosinophils or lymphocytes. Determining the proportion of each type can aid in determining the cause of infection. Heterophils function in phagocytosis, microbicidal activity and inflammatory disease (Strik *et al.*, 2007). Eosinophils are often associated with parasitism and basophils are involved in processing of surface immunoglobulins and releasing histamine, and have also been found in association with blood parasites and viral infection (Strik *et al.*, 2007). Lymphocytes are associated with wound healing, infection and inflammation (Strik *et al.*, 2007). Monocytes are associated with chronic infection, often caused by bacteria or parasites, and azurophils are associated with inflammatory or infectious disease, such as bacterial infection (Strik *et al.*, 2007). We also calculated the heterophil to lymphocyte ratio, which increases in response to stress in snakes (Mader, 2000).

A portion of whole blood was placed in a microhematocrit tube and centrifuged to determine the proportion of red blood cells (PCV) and extract plasma for corticosterone assays. Low PCV can indicate anemia, which can be caused by decreased erythrocyte production, blood loss, viral infection, toxin exposure or starvation (Strik *et al.*, 2007).

In 2007, plasma samples were assayed for corticosterone using a radioimmunoassay following the method described by Wingfield, Vleck & Moore (1992), with inter-assay variation $<18\%$ and intra-assay variation $<5\%$. A small amount of tritiated corticosterone was added to each plasma sample before extracting it, along with the sample corticosterone, into 4 mL of freshly distilled dichloromethane. The organic layer was removed and evaporated under nitrogen at 40 °C, and the sample was re-suspended in phosphate buffer. Each sample was compared with a standard curve of known concentrations, and adjusted based on the

percentage recovery of the added tritiated corticosterone, and to a 1.0 ng mL^{-1} standard included in the assay.

Statistical analyses

We analyzed the effects of radio-transmitters on snake health by comparing snakes with and without transmitters and by comparing snakes before implantation and after carrying transmitters for known durations. For snakes without transmitters, we included snakes that never received a transmitter and snakes sampled only before surgery. If snakes with transmitters were sampled on multiple occasions, we included only one post-surgery sample, chosen randomly.

Hemogregarine loads, WBC concentrations, PCV and corticosterone concentrations were compared between snakes with and without transmitters as well as between snakes with and without ectoparasites using two-sample *t*-tests and ANCOVA (if season was included as a covariate, see seasonal results section). Hemogregarine prevalence relative to transmitter status was assessed using a χ^2 analysis. We compared hematological values before and after transmitter implantation using repeated measures ANOVA. To examine WBC differentials we arcsine transformed the proportion of each WBC type and compared individual and heterophil/lymphocyte ratios between snakes with and without transmitters, as well as between snakes with and without hemogregarines, using two-sample *t*-tests. Although we used transformed data in our analysis, we report proportions in the text. Snake body condition was estimated as the residuals from a regression of snout–vent length on body mass. Although this method may inaccurately estimate condition when individuals vary widely in size (Weatherhead & Brown, 1996), that was not a problem in this study because only adult snakes large enough to carry a transmitter were included in body condition analyses. Body condition was compared between snakes with and without transmitters using ANCOVA with effect of season included as a covariate. We examined all variables for normality, and corticosterone and WBC concentrations were log transformed to increase normality. All means are presented $\pm$ 1 SE.

Results

Blood data

We collected 25 blood samples from 24 individuals in 2006 and 57 samples from 43 individuals in 2007. Nineteen snakes were sampled on multiple occasions, either within a season or between years. Average time between samples was 160.3 ± 29.9 days. None of the hematological characteristics measured differed between male and female snakes (all $P > 0.29$), so sexes were combined for all analyses.

We examined all 82 samples (36 with and 46 without transmitters) for hemogregarines, which were detected in 44% of samples, with a mean of 12.0 ± 4.3 per sample (range = 0–249). Of the snakes sampled multiple times, hemogregarine intensity decreased in subsequent samples for nine individuals (mean 5.4 ± 3.2 fewer), increased for seven (45.9 ± 33.5) and seven had none in any sample.

WBC concentrations for 53 samples (31 with and 22 without transmitters) averaged $354.7/\mu L \pm 35.4$ (range = 32.59–1502.22). We conducted WBC differentials on 80 samples. Lymphocytes comprised the majority of cells ($75.8\% \pm 1.7$), followed by heterophils ($11.0\% \pm 1.6$), azurophils ($4.4\% \pm 0.5$), basophils ($4.4\% \pm 0.4$), monocytes ($3.3\% \pm 0.3$) and eosinophils ($1.2\% \pm 0.2$). PCV for 56 samples (30 with transmitters and 26 without) averaged $25.9\% \pm 1.0$ (range = 8–45%). Corticosterone concentrations in 38 samples (26 with transmitters and 12 without) averaged 4.9 ng $mL^{-1} \pm 0.8$ (range = 0–17.8).

Seasonal variation in snake physiology

Corticosterone concentrations increased through the season ($R^2 = 0.43$, $P < 0.001$, Fig. 1a), whereas body condition decreased ($R^2 = 0.21$, $P < 0.01$, Fig. 1b). This resulted in a negative relationship between corticosterone and body condition ($R^2 = 0.20$, $P = 0.04$). However, residuals of corticosterone regressed on date were unrelated to body condition ($R^2 = 0.08$, $P = 0.20$), so the seasonal corticosterone pattern was probably independent of changes in body mass. The proportion of monocytes ($R^2 = 0.35$, $P < 0.001$), eosinophils ($R^2 = 0.19$, $P < 0.001$) and lymphocytes ($R^2 = 0.12$, $P = 0.01$) all increased through the season. No other variable showed a seasonal pattern (all $R^2 < 0.06$ and $P > 0.07$). Therefore, we included day of year as a covariate in ANCOVA for analyses examining corticosterone, body condition, monocyte, eosinophil and lymphocyte differences between snakes with and without transmitters and parasites.

Effects of transmitters and parasites on snake health

Snakes with and without transmitters had similar hemogregarine prevalence (34 and 65%, respectively; $\chi^2 = 0.25$, $P = 0.62$), hemogregarine intensity (6.6 ± 4.0 and 9.9 ± 5.7, respectively; $t = 0.37$, $P = 0.71$), PCV ($26.4 \pm 2.0\%$ and $28.4 \pm 1.5\%$, respectively; $t = 0.81$, $P = 0.43$, Fig. 2a), proportion of all WBC types (all $P > 0.13$), heterophil/lymphocyte ratios (0.10 ± 0.04 and 0.20 ± 0.05, respectively; $t = 1.38$, $P = 0.17$) and corticosterone concentrations ($F_{1,20} = 0.30$, $P = 0.59$; Fig. 2b). Likewise, snakes with and without transmitters had similar mass (513.5 ± 45.1 and 482.6 ± 31.9 g, respectively; $t = -0.56$, $P = 0.58$) and body condition (-0.2 ± 20.6 and 1.7 ± 14.5, respectively; $F_{1,39} = 0.00$, $P = 0.99$). However, snakes with transmitters had higher concentrations of WBCs than those without transmitters ($t = -2.04$, $P = 0.05$; Fig. 2c).

We also compared snakes before and after they carried a transmitter, and found no significant change in hemogregarine intensity ($n = 14$, 7.1 ± 3.3 and 21.0 ± 17.6, respectively; $F_{1,26} = 0.55$, $P = 0.47$), WBC concentrations ($n = 8$, $F_{1,14} = 0.00$, $P = 0.95$; Fig. 3c), proportion of any WBC type (all $P > 0.13$), heterophil/lymphocyte ratios ($n = 13$, 0.29 ± 0.09 and 0.14 ± 0.04, respectively; $F_{1,13} = 1.60$, $P = 0.23$) or

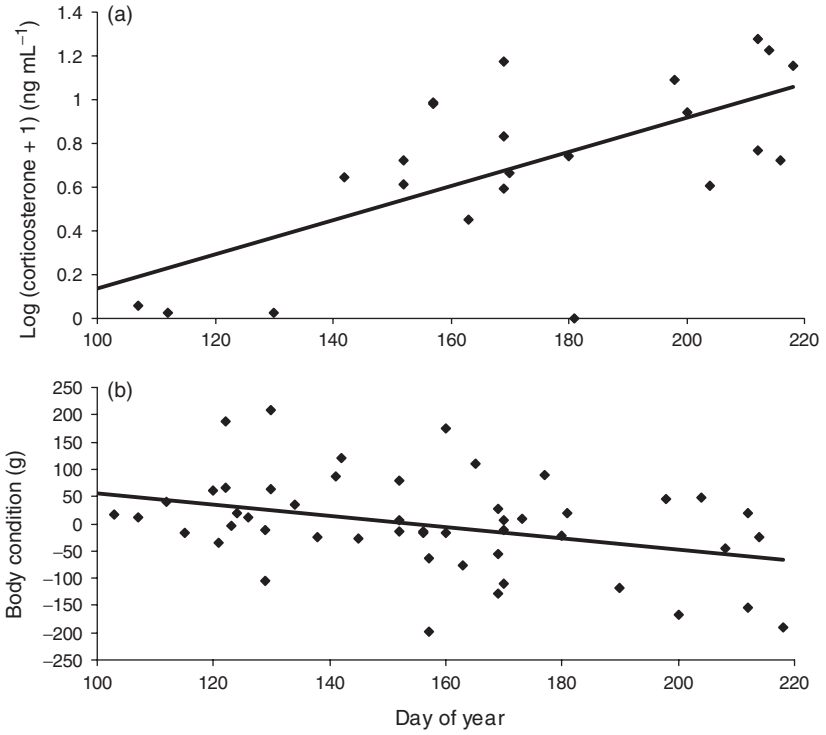

Figure 1 (a) Relationship between total corticosterone (ng mL^{-1}) and day of year in Texas ratsnakes *Elaphe obsoleta* (year 2007). (b) Relationship between Texas ratsnake body condition (g; calculated as residuals of snout–vent length/mass regression) and day of year at Fort Hood, Texas in 2006–2007.

corticosterone concentrations ($n = 5$, $F_{1,8} = 2.08$, $P = 0.19$; Fig. 2b). However, snakes had higher PCV before than after implantation ($n = 9$, $F_{1,9} = 7.63$, $P = 0.03$; Fig. 2a).

Ectoparasites had no significant effect on any hematological characteristics (all $P > 0.07$), whereas snakes with hemogregarines had slightly higher WBC and corticosterone concentrations than those without hemogregarines ($t = -1.34$, $P = 0.19$, Fig. 2c and $F_{1,19} = 3.46$, $P = 0.08$, Fig. 2b, respectively). Among snakes with hemogregarines, only those with transmitters had increased WBC concentrations (Fig. 3a), although the interaction from a two-factor ANOVA was not significant ($F_{1,29} = 2.57$, $P = 0.12$). The same was true of corticosterone: only snakes with both parasites and transmitters had higher corticosterone concentrations (Fig. 3b), but again the interaction was not significant ($F_{1,17} = 1.06$, $P = 0.32$).

Hemogregarines were not associated with differences in proportions of any WBC type (all $P > 0.18$), hematocrit/lymphocyte ratios (with parasites $= 0.14 \pm 0.04$ and without parasites $= 0.19 \pm 0.06$; $t = 0.61$, $P = 0.55$), lowered PCV ($t = -1.49$, $P = 0.14$; Fig. 1a) or snake body condition (with parasites $= 19.5 \pm 16.7$ and without parasites $= -18.2 \pm 16.3$; $F_{1,38} = 2.60$, $P = 0.12$).

Discussion

We found little evidence that either ectoparasites or hemogregarines affected the health of Texas ratsnakes. Some studies of reptiles have found negative effects of parasites (Madsen *et al.*, 2005; Ujvari & Madsen, 2006; Curtis & Baird, 2008), whereas others have found no effect (Caudell *et al.*, 2002; Brown *et al.*, 2006; Schlaepfer, 2006). It is possible that the strength of host response to parasites depends on any additional stressors to which reptiles are subjected. In other taxa there is evidence that multiple stressors can have additive effects (Dallman *et al.*, 1987; McFarlane & Curtis, 1989; McKee & Harrison, 1995). In our study the implantation of radio-transmitters was the equivalent of an experiment in which free-living snakes were challenged with a human-induced burden that may function as an additional stressor. If radio-transmitters compounded the effects of parasites, we would have expected to see differential immune response to parasites in snakes with and without transmitters. We did not find that snakes with transmitters had a higher prevalence of parasitism, but snakes with both transmitters and hemogregarines tended to have higher WBC counts and corticosterone concentrations. Although these differences were not significant, their consistency with the expectation of additive stressor effects suggests that this phenomenon warrants further investigation.

Baseline corticosterone concentrations were higher during the breeding season (late May to July) than before breeding, as occurs in 75% of reptile species studied thus far (Romero, 2002). Because glucocorticoids have so many effects on physiology and behavior (Sapolsky, Romero & Munck, 2000), it can be challenging to identify causes and benefits of seasonal variation in corticosterone (Romero, 2002). In Texas ratsnakes, increased corticosterone during the breeding season appears not to be related to energetic costs of reproduction, because males and females had similar corticosterone levels over time, even though only females bear the cost of egg production. Furthermore,

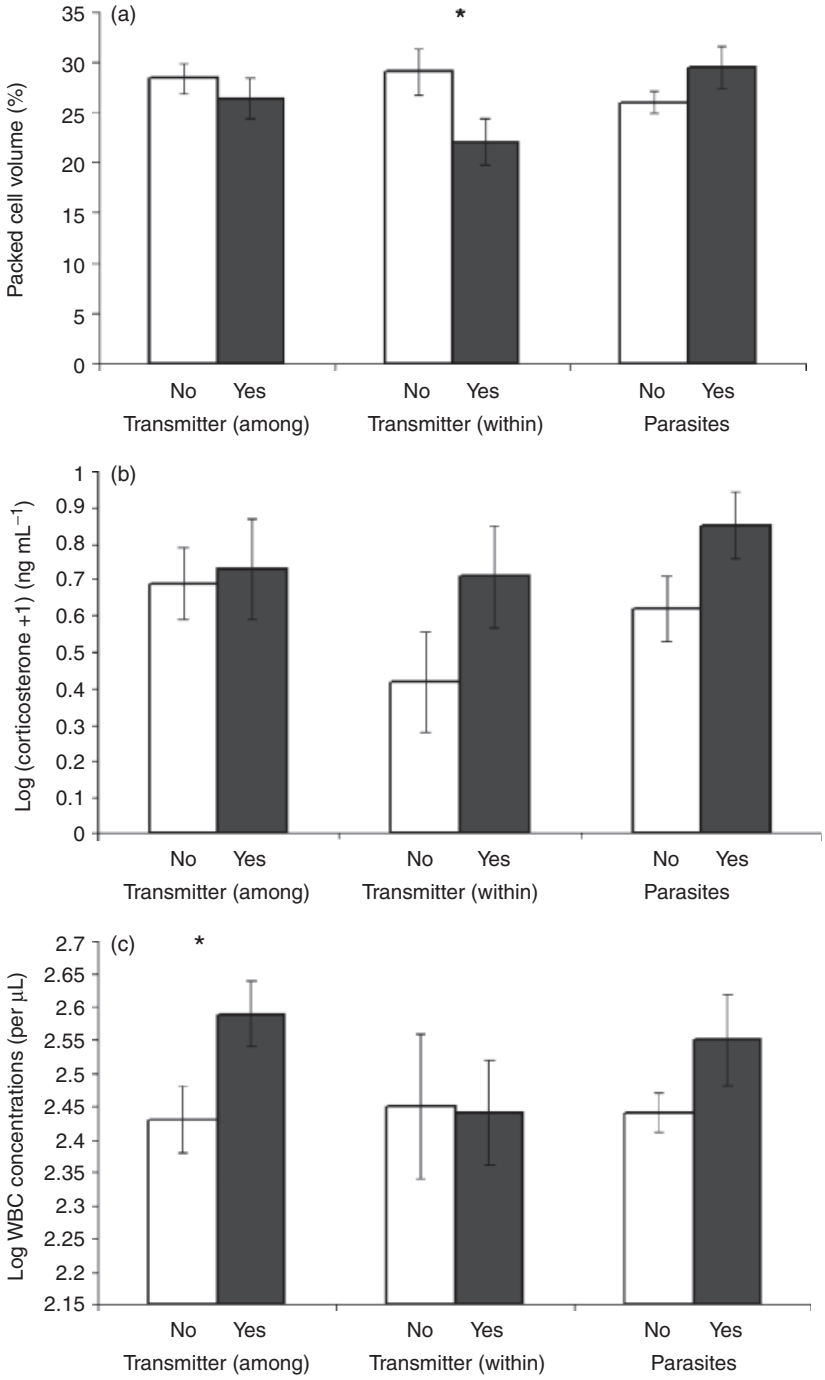

Figure 2 (a) Packed cell volume in snakes with and without radio-transmitters (among individual comparison), before and after transmitter implantation (within individual comparison), and with and without hemogregarine blood parasites. (b) Total corticosterone (ng mL^{-1}) in Texas ratsnakes *Elaphe obsoleta* with and without radio-transmitters, before and after transmitter implantation, and with and without hemogregarine blood parasites. (c) White blood cell (WBC) concentrations in snakes with and without transmitters, before and after transmitter implantation, and with and without hemogregarine blood parasites on Fort Hood, Texas 2006–2007. Asterisks indicate $P<0.05$. All means presented $\pm$ SE.

corticosterone concentrations were unrelated to body condition after controlling for date. However, for both sexes of Texas ratsnakes, the reproductive season coincides with a sharp drop in survival, likely due to predation associated with increased time spent basking and moving (Sperry & Weatherhead, in press). Therefore, corticosterone increases at a time when snakes are at a predictably higher risk of mortality, which supports the idea that seasonal variation in baseline corticosterone reflects differing benefits from the priming role glucocorticoids have in preparing animals to respond to adverse conditions (the 'preparative hypothesis' of Romero, 2002). Importantly, this hypothesis requires only that individuals are at more risk on average during the time of year when corticosterone levels increase – an actual acute stressor, such as a predator attack, is not required (Romero, 2002).

One of our goals was to determine whether carrying radio-transmitters affected the health of ratsnakes, thereby

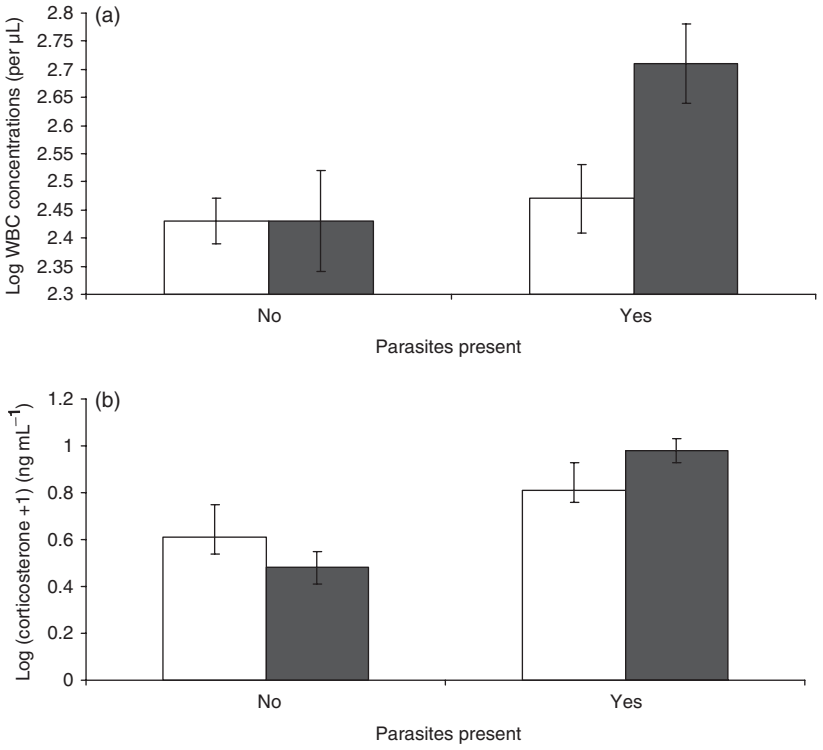

Figure 3 (a) Mean white blood cell concentrations (± SE) in snakes with and without transmitters and hemogregarine parasites on Fort Hood, Texas 2006–2007. (b) Mean total corticosterone concentrations (ng mL^{-1}; ± SE) in snakes with and without transmitters and hemogregarine parasites. White bars indicate snakes without transmitters and black bars indicate snakes with transmitters.

potentially biasing results from telemetry studies. Blouin-Demers & Weatherhead (2001) found a decrease in growth rates and relative clutch mass in black ratsnakes implanted with transmitters, but we found no effect of transmitters on body condition or corticosterone. In fact, for nearly all measures of health examined we found no effect of transmitters. Compared with snakes without transmitters, however, snakes with transmitters did have higher WBC concentrations, but within individuals we detected no change in WBC following transmitter implantation. Similarly, PCV decreased in snakes following implantation but there was no difference between snakes with and without transmitters. Although the two significant transmitter effects were equivocal, it is worth considering what these effects might indicate. Higher leukocyte production could be an indicator of infection, despite surgeries being conducted under sterile conditions. However, the tendency for WBC counts to be higher in snakes with both parasites and transmitters could indicate a response to something other than infection from transmitter surgery. The decline in PCV in snakes after carrying a transmitter could indicate anemia associated with transmitters. Anemia can be caused by decreased erythrocyte production, blood loss, viral infection, toxin exposure or starvation (Strik *et al.*, 2007), although starvation was unlikely because transmitters did not affect body condition.

Overall our results indicate that transmitters had very minor effects at most on the health of Texas ratsnakes. Nonetheless, the possibility that transmitters might affect immune response and erythrocyte function, in conjunction with Blouin-Demers & Weatherhead's (2001) evidence that transmitters negatively affected condition and reproductive effort, argues for further research to explore this issue. Just as for snakes, there is a paucity of data on health effects of transmitters for all taxa. Health effects appear to vary according to surgical methods and study species. Several studies have found no evidence of infection associated with transmitter surgery (e.g. O'Hearn *et al.*, 2005; Moore, Russell & Potter, 2006), whereas others have documented post-surgery infection in at least one study animal (e.g. Knights & Lasee, 1996; Hernandez-Divers *et al.*, 2001; Echols, Vaughan & Moll, 2004). Researchers, particularly those working with endangered species, should consider the implications of using surgically implanted transmitters if infection is a possibility. We recommend that researchers continually monitor health of their study animals and use caution when interpreting results from telemetry studies if infection is suspected. Furthermore, when considering telemetry studies researchers should look for opportunities to design data collection in such a way that assessment of transmitter effects is possible.

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