Copeia, 2007(3), pp. 614–621

Handling, Blood Sampling, and Temporary Captivity Do Not Affect Plasma Corticosterone or Movement Patterns of Gopher Tortoises (*Gopherus polyphemus*)

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Researchers often must capture or trap and physically handle wild animal species to obtain basic morphometric, physiological, or health data. Although these activities affect glucocorticoid levels in many species, few studies have been conducted to determine if they induce changes in animals' subsequent behavior. This is of particular concern to researchers who study Gopher Tortoises (*Gopherus polyphemus*), a threatened species often subjected to trapping and prolonged handling. Therefore, we conducted a study to determine if protocols requiring trapping, handling, blood sampling, injections with innocuous substances, nasal lavages, and temporary captivity affect Gopher Tortoises as indicated by changes in their plasma corticosterone levels, movement patterns, burrow usage, and home ranges. We examined these parameters four weeks prior to and four weeks following implementation of the manipulation protocols (experimental group) or a control date (control group). We found no effect resulting from implementation of the protocol on tortoises' plasma corticosterone levels or movement patterns, including mean distance traveled per move, mean number of days between moves, mean number of burrows used, and home range. The only significant finding was that tortoises in the experimental group showed an increase in the number of times they moved to other burrows from pre- to post-manipulation. However, the slight increase in the number of moves occurred during a time in the season when other studies have also documented increases in movement. Additionally, the increase in number of moves did not change the actual number of burrows used or home range. We conclude that the use of mildly invasive protocols involving short-term procedures and temporary handling do not significantly affect the subsequent corticosterone levels or daily movement patterns of Gopher Tortoises.

IN order to monitor populations of animal species, researchers often capture and physically handle individuals to obtain basic morphometric or physiological data. Handling, though considered fairly innocuous and non-invasive, can initiate a highly conserved stress response in a wide variety of species. For example, in birds it is well established that increased secretion of corticosterone, a glucocorticoid, occurs within minutes of capture and handling (Astheimer et al., 1994; Schwabl, 1995; Romero and Reed, 2005). Other animals that show a similar increase in glucocorticoids as a result of capture and handling include mammals (Widmaier and Kunz, 1993; Morton et al., 1995; Suleman et al., 2004), fish (Fagerlund, 1967; Sumpter et al., 1986), frogs (Coddington and Cree, 1995), turtles (Gregory et al., 1996; Jessop et al., 2004), lepidosaurs (Kreger and Mench, 1993; Tyrrell and Cree, 1998; Jones and Bell, 2004), and alligators (Lance and Elsey, 1999).

In order to avoid the potential effects induced by handling, and to attempt to establish true baseline levels of glucocorticoids, some researchers measure these hormones in ways that eliminate the acts of capturing and handling. One of the most commonly used non-invasive techniques involves the monitoring of fecal metabolites of glucocorticoids. This technique has been used in studies of a wide range of larger species that are difficult to capture and potentially dangerous to handle (Terio et al., 2004; del Castillo et al., 2005; Rolland et al., 2005). However, monitoring of fecal metabolites is confounded by a multitude of other factors (Millspaugh et al., 2001; Millspaugh and Washburn, 2004). Therefore, comprehensive physiological conservation research, which involves collecting data on stress responsiveness, reproduction, health status, and immunocompetence must rely on the use of blood sampling, innocuous injections, and temporary confinement.

The acute increase in glucocorticoids resulting from capture and handling is generally transient, so in many cases, after animals are returned to their habitats, it is generally assumed that they behave in a fashion that is similar to unmanipulated individuals. As a result, little or no follow-up on behavioral patterns is conducted. However, increases in glucocorticoids have been shown to alter sex steroids and may impact

behaviors such as movement patterns and reproductive activities (Greenberg and Wingfield, 1987; Rivier and Rivest, 1991; Pottinger, 1999). Therefore, follow-up is necessary to determine how an animal's subsequent behavior is affected by capture, handling, and manipulation activities.

The impact of mildly invasive research techniques on corticosterone levels and key behaviors is debated by researchers who study Gopher Tortoises (*Gopherus polyphemus*). The Gopher Tortoise is a threatened species that experiences these activities as part of the typical techniques used for conservation actions, such as relocation. At an extreme, these activities might include trapping, handling, blood sampling, injections with innocuous substances, nasal lavages, and temporary captivity. Trapping of Gopher Tortoises, unlike many species, does not cause significant increases in circulating corticosterone levels when they are left in a trap for up to 12 hours (Ott et al., 2000). However, studies have not yet been conducted to determine if more invasive activities than simply trapping the animals may alter corticosterone levels or key behavioral patterns, such as burrow abandonment rates and home range size. Conducting protocols that result in altered movement patterns could be detrimental in the conservation of this species whose habitats and populations have been drastically reduced in size over the last several decades (Auffenberg and Franz, 1982). In this investigation, we examine whether protocols requiring trapping, handling, blood sampling, injections with innocuous substances, nasal lavages, and temporary captivity affect Gopher Tortoises as indicated by changes in their plasma corticosterone levels, movement patterns, burrow usage, or home ranges.

Materials and Methods

Study sites.—Data were collected from adult Gopher Tortoises at the Wade Tract (WT), an 81.5-hectare ecological reserve located on privately-owned land in southwest Georgia (Thomas County) and managed by the Tall Timbers Research Station. The habitat is dominated by nearly pristine, widely spaced, old-growth longleaf pine that provide an open canopy conducive to lush growth of understory plants and the presence of abundant ground level food sources (Johnson, 2004). These qualities, along with well-drained sandy soils and periodic controlled burns to maintain the habitat, create an ideal environment for Gopher Tortoises (Aresco and Guyer, 1999a, 1999b).

Experimental design.—A total of 18 randomly-selected adult, reproductively mature Gopher Tortoises at the WT were examined in this study. There were ten males and eight females ranging in weight from 4.0 kg to over 6.0 kg (exceeding the weight allowed by the digital scale). Six of the 18 tortoises served as controls (five males and one female); they were tracked (described below), but they were not trapped and they did not undergo any type of handling, manipulations, or captivity. From 15–20 June 2002, the remaining 12 tortoises (five males and seven females) were trapped using wire live traps (Tomahawk Live Trap Company, custom order) placed at the mouth of their burrows. The floor and foot pedal of the trip mechanism of each trap were partially covered with sand from the burrow apron. The entrance to the burrow and the trap were covered with a 1-m^2 piece of burlap that provided shade and prevented trapped tortoises from overheating. The traps were set no later than 0800 h and they were checked twice daily until tortoises were caught. Gopher Tortoises do not show an increase in corticosterone when left in a trap for up to 12 hours (Ott et al., 2000), so no tortoise was left in a trap for longer than 12 hours.

Handling and temporary captivity.—Upon capture, the tortoise was removed from the trap and a 1-ml blood sample was immediately drawn from the femoral vein using a 1-ml heparinized syringe and 25-gauge needle. Each tortoise was then placed in an individual 37.85-liter Rubbermaid® bin for transport. The cover and sides of the bin were punctured with numerous 1.5-cm air holes, and the bottom of the bin was filled with 5 cm of sand from the burrow apron. The tortoises were transported to the field lab at Tall Timbers Research Station after all traps were checked (within four hours of the first trap check).

After transport, morphological measurements were taken and each tortoise was subjected to simulated adrenal and actual immune challenges following protocols in Kahn et al. (unpubl. data). First, tortoises were given a 0.1-ml intraperitoneal (IP) injection of saline. At that time, we also gave them a 0.5 ml-subcutaneous injection of 2 mg/ml phytohemagglutinin (PHA) in the ventral webbing of their right medial thigh and a corresponding saline injection in the left thigh. Prior to and 12 hours following the injections, the swelling in each leg was measured to the nearest 0.001 mm using a digital micrometer at the site of injection. Following the saline and PHA injections, we administered a 5-ml injection of 10% unwashed sheep red blood cells (SRBC). Finally, in order to determine if tortoises were

infected with *Mycoplasma agassizii*, the bacterium that causes upper respiratory tract disease (Brown et al., 1999), we flushed 5 ml of 0.9% saline into each naris of the tortoise using a 10-ml syringe with no needle attached. This procedure required that we hold the tortoise's head out of the shell and manually stabilize it behind the occipital bone. At the completion of the manipulations (approximately eight hours after the baseline blood sample was taken), another blood sample was drawn to measure the post-manipulation corticosterone levels. After the 12-hour PHA-induced swelling was measured in the medial thigh, tortoises were returned to their burrows of capture.

As a follow-up, four weeks after undergoing the manipulations protocol, experimental tortoises were trapped again and a blood sample was taken to measure corticosterone. Tortoises were immediately placed back in their burrows of capture. At the completion of the study, all three blood samples from each tortoise were ether-extracted and quantified for corticosterone (ng/ml) using a tritiated steroid radioimmunoassay (Mendonça et al., 1996; Ott et al., 2000) with an intra-assay variability of 10.13%.

Movement.—All 18 experimental and control Gopher Tortoises in this study had been tracked in previous years using radiotelemetry so they already had radiotransmitters attached. We conducted radiotelemetry from 19 May through 14 July 2002, which encompasses the timeframe of four weeks before and four weeks following an individual's trap date, or the 16 June control date for control tortoises. Burrow locations were recorded every two to five days with a GPS and recorded on field data sheets. For each tortoise, we calculated total moves made, mean distance per move, number of burrows used, and home range. In addition, we counted the number of days that passed before tortoises made their first, second, and third moves to other burrows after the control date (control tortoises) or after undergoing manipulations and being placed back in their burrow of capture (experimental tortoises).

Data analyses.—We collected a minimum of four radiotelemetry readings on individual tortoises for four weeks prior to and again four weeks following the trap date for experimental tortoises, or 16 June 2002 for control tortoises. We conducted repeated measures analyses of variance (ANOVAs) to compare pre- and post-manipulation plasma corticosterone levels (experimental tortoises only), movements (total number of moves made from one burrow to another), burrow usage (total number of different burrows used), and home range (95% minimum convex polygon calculated in m^2 using CALHOME; Kie et al., 1996). We also conducted a repeated measures ANOVA to analyze the number of days that had passed (only post-manipulation or control date) before the tortoises left their current burrows and moved to the second, third, and fourth burrows. In addition, we compared movement patterns, burrow usage, and home range for tortoises that underwent physiological manipulations versus those that did not, using one-way and repeated measures ANOVAs. All statistical analyses were conducted with StatView for Windows, Version 5.0.1 (SAS Institute Inc.).

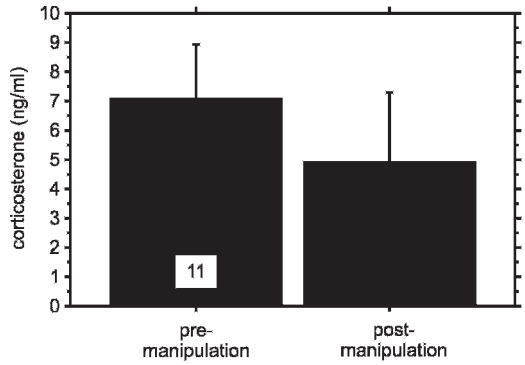

Fig. 1. Mean corticosterone levels (ng/ml) ± SE of experimental tortoises at baseline and approximately eight hours later after undergoing trapping, handling, injections, nasal lavage, and temporary captivity ($F_{1,10} = 0.60$, $P = 0.45$).

Results

Blood samples were collected successfully from 11 of the 12 experimentally manipulated Gopher Tortoises. There was no significant difference in tortoises' corticosterone levels from pre- to post-manipulation ($F_{1,10} = 0.60$, $P = 0.45$, Fig. 1). Immediately after tortoises were removed from the traps, they had a mean of 7.10 ng/ml of corticosterone. Approximately eight hours later, after the tortoises experienced handling, manipulations, and temporary captivity, they had a mean of 4.90 ng/ml of corticosterone. As a follow-up, ten of the 12 tortoises were trapped and blood sampled again four weeks after they underwent manipulations. There was no significant difference in their corticosterone levels between the baseline measure taken prior to the manipulations (mean ± SE, 7.10 ng/ml ± 1.80) and at the 30 day measure (8.23 ng/ml ± 1.50, $F_{1,10} = 0.30$, $P = 0.59$).

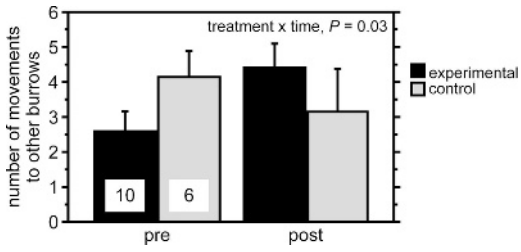

Fig. 2. Mean number of movements made ± SE to other burrows by tortoises during the four weeks prior to and four weeks following implementation of the protocol (experimental group) or control date (control group). (Interaction effect treatment x time, $F_{1,14} = 5.30$, $P = 0.03$.)

Overall, the movement measures did not show any significant differences from pre- to post-manipulation (or control date) or between experimental and control groups. Specifically, in the group that underwent manipulations, there was no significant difference in the mean distance that tortoises moved before and after the manipulations were conducted ($F_{1,9} = 0.60$, $P = 0.45$). In addition, the control group showed similar patterns (i.e., no significant difference in mean distance moved) before and after the control date ($F_{1,5} = 0.08$, $P = 0.78$). There was also no significant difference between the experimental and control groups in the number of days that passed between the first, second, and third moves to other burrows after the manipulation or control date ($F_{1,10} = 0.12$, $P = 0.73$), or in the actual number of days that passed between making those moves ($F_{2,10} = 0.81$, $P = 0.45$).

The total number of burrows that tortoises used before and after the protocol administration or control date was not significantly different ($F_{1,14} = 2.75$, $P = 0.12$), regardless of treatment group ($F_{1,14} = 0.11$, $P = 0.74$). Furthermore, tortoises did not show a significant change in home range size from pre- to post-manipulation or control date ($F_{1,14} = 0.73$, $P = 0.41$), regardless of treatment ($F_{1,14} = 0.20$, $P = 0.65$).

There was no significant difference between the experimental and control groups in the number of movements tortoises made during the four weeks before and four weeks after the protocol was implemented or control date ($F_{1,14} = 0.03$, $P = 0.86$). However, there was an interaction effect for treatment x time ($F_{1,14} = 5.30$, $P = 0.03$, Fig. 2). When analyzed separately by treatment, we found that experimental tortoises that were subjected to the protocol moved significantly more frequently during the four weeks following administration of the protocol as compared to the four weeks prior to implementation of the protocol (post vs. pre, mean number of moves ± SE: 4.4 ± 0.65 vs. 2.6 ± 0.56, $F_1 = 5.87$, $P = 0.04$). Control tortoises showed no change from pre- to post-control date in the number of moves they made ($F_{1,9} = 1.07$, $P = 0.35$).

Discussion

Conducting physiological examinations of threatened and endangered species through procedures that are considered by some to be invasive has been shown to increase corticosterone levels in many species and is often thought to potentially disrupt an animal's normal behavior patterns. However, we found that Gopher Tortoises do not experience a change in corticosterone levels and they do not change their behavior in response to trapping, handling, blood sampling, injections with innocuous substances, nasal lavages, and temporary captivity. There are numerous studies indicating that turtle species experience acute handling stress via a significant glucocorticoid response (Gregory et al., 1996; Gregory and Schmid, 2001; Jessop et al., 2004). However, there are also other studies using several reptile models that support our finding and demonstrate that mildly invasive research activities do not affect study animals in terms of glucocorticoid levels. For example, in a previous study, Kahn et al. (unpubl. data) found no significant difference in Gopher Tortoises' corticosterone levels when comparing samples from the initial trap date and one, three, 11, 32, and 52 days in captivity, during which time tortoises also underwent physical manipulations, including those conducted in this study. In addition, the Bearded Dragon (*Pogona barbata*) shows no significant change in corticosterone levels in captivity at either 3.5 or 24 hours post capture (Cree et al., 2000). Three-Toed Box Turtles (*Terrapene carolina triunguis*) also do not experience a significant increase in fecal glucocorticoid metabolite levels when subjected to capture, handling, attachment of a radiotransmitter, and temporary captivity (Rittenhouse et al., 2005), indicating that this species does not experience a stress response as a result of these activities. Similar findings were documented in mammal species. Koalas do not show an increase in plasma cortisol levels at six hours post capture or at one or seven days in captivity (Hajduk et al., 1992). In addition, African Wild Dogs (*Lycaon pictus*), when compared with control animals, do not show increases in fecal glucocorticoids or

increased risk for mortality when they are tranquilized with a dart and radiocollared (Creel et al., 1997). It appears that, at least in the short-term, these mildly invasive manipulations have little or no effect on the glucocorticoid levels of the individuals being studied. The procedures we conducted were both more extensive and more invasive than those conducted in any of these studies, and yet, we also demonstrated no change in Gopher Tortoises' corticosterone levels as a result of our protocols.

None of the key behavioral variables that we studied, except for one, showed any significant differences between experimental and control animals or from pre- to post-manipulation or control date. These findings are similar to results of another recent study that demonstrated no differences in recapture rates or time to recapture between groups of Gopher Tortoises that were previously captured, handled, and marked versus those that were not (Pike et al., 2005). The only significant finding in this study was an interaction effect between treatment and time in the number of movements tortoises made to other burrows during the four weeks following the manipulation or control date. Despite this finding, tortoises remained within the same home range and continued to use the same burrows. In addition, increased movement during the post-manipulation period, as demonstrated by the experimental group, is also seen in other Gopher Tortoise populations. Specifically, increased movement rates occur later in the active season, usually from July through September as the mating season progresses (McRae et al., 1981; Eubanks et al., 2003). Given these results, it appears that the increase in the experimental group was more typical of Gopher Tortoise behavior than what we observed in our controls.

In general, the movement patterns that we documented are similar to those reported in other studies of Gopher Tortoises. For example, Eubanks et al. (2003) conducted a study of Gopher Tortoises at the Joseph W. Jones Ecological Research Center, a private ecological reserve in southwest Georgia (Baker County) with habitat similar to that of the Wade Tract. They found that female and male tortoises traveled mean distances of 54.0 m and 85.2 m per move, respectively, from June through October 1997. We found a mean distance of 76.0 m per move prior to the manipulations and 52.2 m per move after the protocol was implemented, which are in the same general range and the same general time period as the Eubanks et al. (2003) study. Our control tortoises had mean distances traveled of 42.8 m per move prior to the control date and 37.6 m per move afterwards. Thus, it appears that our experimental tortoises, but not our control tortoises, traveled mean distances that were comparable to the findings of this other study.

The mean number of burrows that tortoises used in the course of our study did not change significantly between pre- and post-manipulation or control date. Experimental tortoises used a mean of 2.4 burrows prior to manipulation and 3.7 burrows post-manipulation, whereas control tortoises used 3.1 and 3.3 burrows pre- and post-control date, respectively. These results are similar to those from other Gopher Tortoise studies. Again, Eubanks et al. (2003) found that female tortoises used an average of about two burrows per month throughout the year, whereas males increased the number of burrows they used from May through September, using a maximum of about five burrows. At another location in Georgia, McRae et al. (1981) documented similar findings to those of the Eubanks et al. (2003) study. They found that tortoises changed burrows infrequently in the early part of the active season, but by summer, tortoises began using two or more burrows per month.

The home ranges that we documented in this study were smaller than those documented in other studies. However, our study was conducted for a period of only eight weeks and most studies report figures for home range over the course of an entire season or year. For example, prior to the manipulation or control date, our tortoises (males and females combined) had a mean home range of 0.05 hectares for the experimental group and 0.17 hectares for the control group. In similar habitat, Eubanks et al. (2003) calculated average annual home ranges for females and males to be 0.4 hectares and 1.1 hectares, respectively, which are very different from our findings. Similarly, in two Florida studies that were conducted over the course of two years, researchers reported annual home ranges for Gopher Tortoises to be 0.6 and 0.31 hectares for females and 1.9 and 0.8 hectares for males (Diemer, 1992; Smith et al., 1997). The home range data that we report are more comparable to the McRae et al. (1981) study in Georgia, where female and male tortoises had home ranges 0.08 hectares and 0.47 hectares, respectively. Perhaps our home ranges are smaller than those reported in most studies due to the abbreviated time frame of our study (spring and early summer months, prior to reported increases in male movement patterns). In fact, we found that in May/June, prior to the manipulations or control date, nine out of 16 tortoises (56%) used only one or two burrows, and a total of only four

tortoises (25%) used only one or two burrows post-manipulation or control date (June/July). As a result of these small numbers in burrow usage, the home ranges we report are also small. Ultimately, our data analyses indicate that manipulations had no effect on home range, and we do not think there will be an alteration to the home ranges if examined over the course of an entire season of activity.

In general, few studies have examined direct links between behavior and handling as a stressor. However, studies that did attempt to examine such a link supported our findings that these mildly invasive techniques may not be stressors. Specifically, one study showed that a common territorial lizard, *Anolis sagrei*, does not increase display behaviors in response to investigator handling and temporary confinement, indicating that the research activities are not stressors (McMann and Paterson, 2003). Another study found that despite an increase in corticosterone as a result of four hours of capture stress, male Red-Sided Garter Snakes (*Thamnophis sirtalis parietalis*) do not alter their mating behavior relative to controls (Moore et al., 2000).

The information gained from conducting mildly invasive research techniques may be crucial to the survival and conservation of Gopher Tortoises. The procedures we conducted in our study were both more extensive and more invasive than the procedures conducted in many other studies of this species, and yet, we demonstrated no change in Gopher Tortoises' physiological or key behavioral variables as a result of our protocols. Ultimately, our study provides evidence that these short-term procedures do not significantly affect the corticosterone levels or daily movement patterns of Gopher Tortoises.

Acknowledgments

We thank the United States Fish and Wildlife Service and the National Council for Air and Stream Improvement for funding this project. This material is based upon work supported by the National Science Foundation under Grant No. EPS-0447675. All protocols were approved by the Auburn University Institutional Animal Care and Use Committee, PRN numbers 0303-R-2534 and 2003-0468. We also extend our sincere appreciation to R. Birkhead for collecting data at the Wade Tract and for sharing his knowledge and expertise of Gopher Tortoises. Finally, we thank the property owners, Mr. and Mrs. J. Wade, for the generous use of their lands and Tall Timbers Research Station for logistical support in completing this project.

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