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Effects of Testosterone and Training on Performance in the Non-Territorial Lizard (Aspidoscelis Sexlineata)

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EFFECTS OF TESTOSTERONE AND TRAINING ON PERFORMANCE IN THE NON-TERRITORIAL LIZARD (*ASPIDOSCELIS SEXLINEATA*)

by

Jennifer L. O'Connor

Under the direction of Lance D. McBrayer

ABSTRACT

In territorial lizards, testosterone (T) has been suggested as a possible mediator of seasonal increases in endurance and bite force, which are important for maintaining territories and winning male-male competitions. *Aspidoscelis sexlineata*, a non-territorial lizard, has elevated bite force and endurance during the breeding season when T levels are elevated (Gowan 2008). Here, I investigated the effects of experimentally elevated T levels on bite force and endurance. Tonic-release implants elevated T levels for 2 weeks; however, they failed to increase performance during that time. Endurance decreased for all lizards in the lab study; suggesting that captivity negatively affects endurance. A second study was then conducted to examine the effects of training and T supplementation on locomotor performance in *A. sexlineata*. Training did not increase locomotor performance of male *A. sexlineata;* however, elevated T increased percent hematocrit. Ventral hue and testis volume decreased for all males in the second lab study, which may have been the result of chronic stress or the absence of environmental cues needed to induce or maintain breeding season condition. This study provides insight into the morphological and physiological traits under the influence of T in non-territorial species.

INDEX WORDS: *Aspidoscelis sexlineata*, implant, bite force, locomotor performance, testosterone

EFFECTS OF TESTOSTERONE AND TRAINING ON PERFORMANCE IN THE NON-TERRITORIAL LIZARD (*ASPIDOSCELIS SEXLINEATA*)

by

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CHAPTER 1

EFFECTS OF TESTOSTERONE ON BITE FORCE AND ENDURANCE IN THE SIX-LINED RACERUNNER (*ASPIDOSCELIS SEXLINEATA*)

ABSTRACT

Numerous studies have examined the effects of testosterone (T) on behavior in territorial lizards, but few have explored the role of T in non-territorial lizards. Thus, I quantified the morphological and physiological factors affecting performance in *Aspidoscelis sexlineata* and I provide valuable information concerning how T affects non-territorial mating systems. I experimentally elevated T levels in a common non-territorial lizard found throughout much of the southeastern United States. Adult male *A. sexlineata* were captured in Ocala National Forest in Florida between April-Sept. 2008. Blood samples were collected upon capture and later analyzed for T concentration. Lizards were returned to the laboratory where bite force and endurance were tested. After initial performance trials, lizards were given either a T implant or empty implant. T levels, morphological traits, bite force, and endurance were measured biweekly for 4 weeks. At the end of the experiment, testis volume and the mass of several hindlimb muscles were measured. T implants elevated T levels within the normal physiological range for 2 weeks, but did not increase bite force or locomotor performance during this time. Endurance decreased for all lizards in the breeding and post-breeding season experiments suggesting that captivity may have negatively impacted endurance. This study is the first to examine the effects of experimentally elevated T levels on bite force and endurance of a non-territorial lizard.

INTRODUCTION

Locomotor performance affects food acquisition, mate acquisition, and predator avoidance. Animals with greater locomotor performance (i.e. sprint speed) are better able to escape from predators thereby increasing their chances of survival (Jayne and Bennett 1990). Furthermore, males with elevated sprint speeds may have increased reproductive success because of their ability to defend larger territories and mate with more females (Husak et al. 2006). Differences in morphology (i.e. hindlimb length, Husak et al. 2006) can account for a considerable amount of variation in locomotor performance. However, physiological factors also influence performance (Arnold 1983, Garland 1994, Garland and Losos 1994). Testosterone, an anabolic steroid, fluctuates in males with seasonal breeding cycles. For many males, T levels peak just before or during the breeding season, when testes are maximal in size (Johnson and Jacob 1984). In males, T influences a suite of traits associated with breeding. For example, several studies have cited T as a possible mediator of seasonal increases in bite force (Irschick et al. 2006, Husak et al. 2007, Gowan 2008) and sprint speed (Garland et al. 1990, Klukowski et al. 1998) associated with the breeding season. Additionally, males with higher levels of T have increased activity levels (DeNardo and Sinervo 1994, Wikelski et al. 1999) and defend larger territories (Sinervo et al. 2000, Cox et al. 2005).

In non-territorial lizards, competition for resources (i.e. food, favorable habitats, and mates) may still result in agonistic interactions. For *Aspidoscelis sexlineata*, a common non-territorial lizard, agonistic interactions between males are not uncommon during the breeding season (Fitch 1958, Hardy 1962). Often, larger males are observed chasing and biting smaller males, which are often displaced from the more favorable habitats (Fitch 1958, Hardy 1962). Some chases are extremely short (Bellis 1964), while others may last up to 30 m in length (Carpenter 1960). In captivity, males exhibit the same agonistic behaviors (i.e. chasing and biting) that have been observed in the wild (Brackin 1978). However, these agonistic behaviors only occur during the breeding season (Brackin 1978). Elevated T levels have been suggested as a proximate cause of increased aggression levels in male *A. sexlineata* (Hardy 1962). Similar to other temperate lizard species, T levels vary seasonally in male *A. sexlineata* (Johnson and Jacob 1984, Grassman and Hess 1992a, Gowan 2008), with T levels being greatest during the breeding season (Johnson and Jacob 1984, Grassman and Hess 1992a). For many species studied to date, winners of these male-male competitions have increased fitness (Arnold 1983), and it is likely that the same is true of *A. sexlineata*.

Corticosterone (B), a steroid hormone, is involved with the stress response of many animals, and is often studied in connection with locomotor performance. In territorial lizards, B levels often increase following social encounters (Smith and John-Alder 1999). Body size may also influence the relationship between B and T levels (Husak et al. 2007). For example, plasma T and B levels are negatively correlated in smaller male *A. carolinensis*, but no relationship exists between B and T in larger males (Husak et al. 2007). In field-active male *A. sexlineata*, plasma B and T follow the same seasonal pattern, with B and T being elevated during the breeding season (Grassman and Hess 1992a). In a lab study, Brackin (1978) found that smaller male *A. sexlineata* had elevated B levels (evidenced by adrenal hypertrophy) compared to larger males during the breeding season. Although the seasonal profiles of B and T are known for male *A.*

sexlineata, the affect of these hormones on performance is unknown. Hence, steroid hormones may influence performance in non-territorial lizards.

During the breeding season, non-territorial males spend the majority of their activity periods searching for food and mates. Because the home ranges of non-territorial males greatly overlap, encounters are common during the breeding season when activity levels are greatest (Fitch 1958, Clark 1967, Gowan 2008). The effects of T on territorial lizards have been studied extensively (see Cox et al. 2009 and references therein). However, the effects of T on non-territorial lizards have not. The focus of the present study is to answer the following questions: (1) Do experimentally elevated T levels (within the normal physiological range for *A. sexlineata*) increase bite force and endurance during the breeding season? (2) Can T implants elevate bite force and endurance in post-breeding season males to equal those of breeding season males? (3) Do T implants increase the mass of hindlimb muscles involved in locomotor performance? I predict that experimentally elevated T levels will result in increased bite force and endurance. By experimentally elevating T levels in post-breeding season males, I predict that bite force and endurance will be elevated to that of breeding season males. Because T supplementation increases muscle mass (Bhasin et al. 2001), I predict that hindlimb muscle mass of T-implanted males will be greater than that of empty-implanted males.

MATERIALS AND METHODS

Study species

Whiptail lizards of the genus *Aspidoscelis*(=*Cnemidophorus*) are quick moving, active foragers found throughout much of the United States and Mexico (Wright 1993).

Aspidoscelis sexlineata is a non-territorial, bisexual species found throughout the southeastern United States (Ballinger et al. 1979, Conant and Collins 1998). Adults have an active season lasting approximately 5 months (from late April-early Sept., Etheridge et al. 1983, Johnson and Jacob 1984). Home ranges of *A. sexlineata* average approximately 13,000 m^2 in size (Clark 1967), and males occur in densities of 15-24/ ha (Clark 1967). Male and female *A. sexlineata* are essentially the same size (in snout-vent length, Bellis 1964). However, males have larger heads than females and males have a bright blue ventral coloration (Fitch 1958), which extends up onto their sides, making them easy to distinguish from females at a distance of several meters (personal obs).

Adult male *A. sexlineata* (SVL $\geq$ 54 mm, Hoddenbach 1966) were captured at four sites in Ocala National Forest, Florida, between May-Sept. 2008. The majority of searching effort was focused along jeep trails and at the edges of sand pine scrub habitats or longleaf pine habitats. All study sites contained large areas of open sand, which is considered ideal habitat for *A. sexlineata* (Greenberg et al. 1984). Sampling was restricted to 0900-1400 h to minimize any diurnal fluctuations in hormone levels.

To minimize any increases in corticosterone levels as a result of elevated stress levels, lizards were captured by noose within 5 min of being seen. If an individual was pursued for more than 5 min, it was not captured. Also, lizards missing more than one-third of their tail were not captured because tail loss negatively affects running performance (Ballinger et. al. 1979). Sex was confirmed by everting the hemipenes and blood (approximately 50-100 µL) was collected from the post-orbital sinus within 5 min of capture using a heparinized capillary tubule (Fisherbrand, Cat. No. 22-362-566; MacLean et al. 1973, Grassman and Hess 1992b). Blood samples were centrifuged immediately on site then stored on dry ice until being returned to the laboratory where they were stored at -20°C. Lizards were kept in cloth holding bags inside a cooler (to minimize extreme fluctuations in temperature) and returned to the laboratory.

Housing

Adult male *A. sexlineata* (n = 29 in the breeding season, n = 29 in the post-breeding season) captured between May-Sept. 2008 were maintained in the laboratory for the implant portion of the study. Lizards were housed separately in glass aquariums (dimensions: 50.8 x 27.9 x 33.0 cm^3) with a sand substrate. Cardboard inserted between aquariums prevented lizards from seeing one another and thus prevented any agonistic interactions. Water was always available and lizards were fed (crickets dusted with Reptocal® and mealworms) until satiation three times per week. A single 100W incandescent bulb suspended over one end of the aquarium created a basking spot and an average temperature gradient of 34-41°C along the length of the aquarium. Automatic timers controlled lighting, and photoperiod was adjusted according to season (11L: 13D for breeding season, 10L: 14D for post-breeding season). A source of UVB (Zoo Med, 40W Reptisun bulb) was provided daily. Lizards were maintained in the laboratory for approximately 7 wks.

Implants

Lizards captured during the breeding season and post-breeding season were randomly assigned to a treatment group: (control) empty implant (n = 14 in the breeding and post-breeding season experiments) or T implant (n =15 in the breeding and post-breeding season experiments, Fig. 1.1). Implants were constructed out of 4-mm length sections of Silastic® tubing (Dow Corning, Cat. No. 508-006, 1.47 mm ID x 1.96 mm

OD) following the methods of Cox et al. (2005). One end of the implant was closed with silicone sealant and cured for approximately 2 h. Using a Hamilton® syringe, 3 μL of a solution containing T (Sigma, T-1500) dissolved in dimethyl sulfoxide (DMSO) at a concentration of 100 μg T/μL of DMSO (Cox et al. 2005) was injected into each T implant. The open end of each implant was sealed with silicone sealant. Empty implants were filled with 3 μL of DMSO and closed with silicone sealant. Implants were placed under a fume hood for 5 d to allow the DMSO to diffuse out of the implants.

Prior to surgery, lizards were anesthetized with an intramuscular injection of 2 % ketamine. A small incision was made in the right lateral fold of the lizard (slightly anterior to the hindlimb) and either an empty or T implant was inserted subcutaneously. The incision was sealed with Vetbond tissue adhesive (3M). Lizards recovered from the surgery for 5 d before performance trials resumed. Blood samples were collected from the post-orbital sinus every 2 wks to confirm that T implants were releasing hormone.

Hormone Analysis

Plasma T concentrations were measured using an enzyme immunoassay kit purchased from Cayman Chemical Co., Ann Arbor, MI (#58270). In the assay, an unknown concentration of T (in plasma samples) competes with a known amount of T tracer for a limited number of binding sites. Due to small plasma volumes (10-30 μL), the plasma to buffer ratio was adjusted for each sample. For example, the amount of buffer added to each un-extracted sample was determined based on if the sample was collected in the breeding season or post-breeding season and the volume of plasma present. Samples collected during the breeding season were diluted more than samples collected during the post-breeding season (i.e. 300-500 μL of buffer versus 100-200 μL of buffer). Next, 50 μL of the plasma + buffer solution was pipetted into each well. All plasma samples were run in duplicate. Then, a constant amount of tracer and antiserum were added to each well. The microtiter plate was covered with plastic film and developed in the dark for 2 h on an orbital shaker. Once the plate had developed, it was rinsed with wash buffer five times to remove any unbound reagents. Then, Ellman's Reagent was added to each well of the plate. The plate was covered with plastic film and developed for 30 min, at which point the plate was read using a microplate autoreader (Bio-Tek instruments, EL311s) at wavelength of 405 nm. The microplate autoreader produced a table of absorbance values, which were entered into an electronic spreadsheet provided by Cayman Chemical Co. A standard curve (made from known T concentrations) was plotted for the assay. A linear regression using the standard curve and absorbance values allowed the unknown T concentrations of the plasma samples to be calculated. The concentration of T tracer was inversely proportional to the amount of T in each sample. The mean T concentration for each lizard was calculated, and only samples that fell within the sensitive range of the standard curve (20-82% bound analyte) were retained for statistical analyses. Several lizards collected in the post-breeding season had T levels that fell below the sensitivity of the curve. Therefore, these samples were not included in the statistical analyses. For the T assays, the inter-assay variation was 19.94 % and the intra-assay variation was 16.91%.

Blood samples from males in the post-breeding season experiment were also analyzed for B concentrations. Blood samples of males in the breeding season experiment were not analyzed due to a limited number of blood samples remaining after T analysis. A radioimmunoassay (RIA) was used to quantify B concentrations following the methods of Wingfield and Farner (1975). The mean extraction efficiency was 91% and intra-assay variation was 12%.

Measurement of performance

In the lab, bite force and running endurance were tested. All performance trials were conducted between 0900-1400 h. Lizards were fasted for 12 h before performance trials since feeding negatively affects endurance (Huey et al. 1984). Before each trial, lizards were placed in a 40°C incubator for approximately 30 min to ensure that they reached their field-active body temperature (38-41°C, Hardy 1962). All body temperatures were confirmed with a cloacal thermometer. Bite force was tested following the methods described by McBrayer and Anderson (2007) and Gowan (2008). Three trials were conducted on the same day (with 1 min rest between trials) and the greatest value was retained for statistical analysis. Bite force was tested first (i.e. Day 1) followed by three consecutive days of endurance testing.

Endurance was measured on 5-m circular track with an artificial turf substrate. At the beginning of the trial, the lizard was chased at full speed with a small broom. Once the lizard's speed slowed to less than a sprint, taps on the tail were used to motivate the lizard to continue its forward motion. Every 30 s, the total distance covered by the lizard was recorded. The trial ended when the lizard failed to move forward after ten consecutive taps on the tail and the lizard had lost its righting response (Garland 1984, Cullum 1997). If the lizard was able to right itself, the trial continued until the lizard reached exhaustion. The fastest trial (in which the lizard covered the greatest distance in the shortest amount of time) was retained for statistical analysis. Bite force and endurance were tested again at 2 wks and 4 wks post-implant in the breeding and post-breeding season experiments. Endurance data are reported as maximum time run (s).

Measurement of morphology

Pre-treatment and post-treatment values for snout-vent length (SVL) and mass were recorded for all lizards. Upon completion of the performance testing (after 7 wks in the lab), lizards were euthanized with an overdose of sodium pentobarbital. Specimens were fixed in 10% buffered formalin; then preserved in 70% ethanol (EtOH). Because T may influence muscle size, several hindlimb muscles were isolated, weighed and measured. The diameter of the following hindlimb muscles was measured to the nearest 0.1 mm using dial calipers before being dissected free: iliofibularis (IF), iliotibialis (IT), ambiens ventralis (Amb V), flexortibialis internus (FTI), puboischiotibialis (PIT), gastrocnemius minor (G minor) and gastrocnemius major (G major). Upon removal, each muscle was soaked in EtOH for 5 min, blotted dry on filter paper, and weighed to the nearest 0.1 mg on an electronic balance (Ohaus Adventurer Pro AV64). To calculate testis volume, testis length and width was measured to the nearest 0.1 mm using a dissecting microscope equipped with an ocular micrometer. Testis volume was determined by calculating the volume of an ellipsoid ($V = 4/3\pi a^2 b$; following Mayhew 1963) and the average testis volume for each lizard was retained for statistical analysis.

Statistical Analyses

Two-way ANOVAs were used to test for the effects of season and exogenous T on testis volume, muscle mass, and muscle diameter. The following measurements were $\log_{10}$ transformed to meet the parametric assumption of normality: corticosterone concentrations (ng/mL), T concentrations (ng/mL), mean testis volume (mm^3), maximum bite force (N), maximum time run (s), and mass (g). A Kruskal-Wallis test was used to compare treatment groups when data could not be transformed to meet the parametric assumptions. A linear regression of bite force on SVL revealed that lizard size accounted for a large amount of variation in bite force ($r^2 = 0.445$, $P < 0.001$). Therefore, size-free residuals were calculated and used in subsequent analyses. A repeated-measures ANOVA was used to analyze changes in hormone levels and performance variables between treatment groups over the course of the experiment. All statistical analyses were performed using JMP ® (version 7.0.1). Significance was accepted at $P < 0.05$, and all P values are two-tailed.

RESULTS

Field Data

Lizards captured during the breeding season and post-breeding seasons did not differ in SVL (T-test, T = 1.88, df = 47, P = 0.066). Additionally, there was no difference in bite force or maximum time run among sampling sites within Ocala National Forest during the breeding season (ANOVA, maximum time run: $F_{3, 23} = 1.24$, P = 0.318, bite force: $F_{3, 23} = 0.60$, P = 0.625) or post-breeding season (maximum time run: $F_{4, 22} = 0.36$, P = 0.836, bite force: H = 2.84, P = 0.585). Plasma T levels and maximum time run were greater in the breeding season than in the post-breeding season (Mann-Whitney U Test, testosterone: U = 92, P = 0.039, $N_1 = 11$, $N_2 = 11$, Fig. 1.2; maximum time run: $F_{1, 52} = 41.999$, $P < 0.001$, Fig. 1.2). Bite force was not different between seasons ($F_{1, 52} = 0.33$, P = 0.565, Fig. 1.2).

Implants

Initial plasma T levels from samples collected in the field were not different between treatment groups (i.e. control or T implant) in the breeding season or post-breeding season experiment (ANOVA, breeding: $F_{1, 10} = 2.28$, $P = 0.162$; post-breeding: $F_{1, 10} = 0.38$, $P = 0.555$). At 2 wks post-implant, plasma T levels of T-implanted males were elevated compared to those of empty-implanted males (breeding: Mann-Whitney U Test, $U = 55$, $P = 0.002$, $N_1 = 11$, $N_2 = 5$; post-breeding: ANOVA, $F_{1, 6} = 12.59$, $P = 0.012$). T implants successfully elevated plasma T levels within the normal physiological range of *A. sexlineata* (less than 1 ng/mL - 20 ng/mL, Johnson and Jacob 1984, Grassman and Hess 1992a) for 2 wks during the breeding season and post-breeding season experiments. At 4 wks post-implant, plasma T levels of T-implanted males were not significantly different from initial T concentrations.

Morphology

Hindlimb muscle masses were not significantly different between treatment groups in the breeding season or post-breeding season experiment. There was a significant interaction of implant type and season on the diameter of the ambiens ventralis ($F_{1, 18} = 5.2481$, $P = 0.0343$). The diameter of the ambiens ventralis was greatest in T-implanted males in the breeding season experiment (Tukey-Kramer, $p < 0.05$). However, there was no effect of season or implant on the diameter of the ambiens ventralis ($F_{1, 18} = 1.3661$, $P = 0.2577$, $F_{1, 18} = 0.0197$, $P = 0.8900$, respectively). No other differences in muscle masses or muscle diameters were detected between treatment groups in either the breeding season or post-breeding season experiments. Finally, to determine if a change in total hindlimb muscle mass had occurred, the mass of all hindlimb muscles (that were dissected free) were summed. In the breeding season experiment, hindlimb muscle masses of T-implanted males seemed to increase compared to that of empty-implanted males although this trend was not significant ($F_{1, 10} = 3.55$, $P = 0.089$). Total hindlimb muscle mass was not significantly different between treatment groups in the post-breeding season experiment ($F_{1, 8} = 0.003$, $P = 0.958$).

Season had a significant effect on testis volume ($F_{1, 33} = 15.79$, $P < 0.001$). Empty-implanted males had smaller testes in the post-breeding season experiment than in the breeding season experiment (Tukey-Kramer, $p < 0.05$, Fig. 1.3). However, T-implanted males had similar-sized testes in the breeding season and post-breeding season experiments (ANOVA, $F_{1, 15} = 2.82$, $P = 0.114$, Fig. 1.3). The testis volume of empty-implanted males was larger than that of T-implanted males during the breeding season experiment (ANOVA, $F_{1, 23} = 4.62$, $P = 0.042$, Fig. 1.3).

Performance

Endurance decreased significantly over time for all implanted males during the breeding season and post-breeding season experiments (repeated-measures ANOVA, breeding: $F_{2, 22} = 17.05$, $P < 0.001$, post-breeding: $F_{2, 20} = 9.76$, $P = 0.0011$, Fig. 1.4). Implant type had no effect on endurance in the breeding season or post-breeding season experiment (repeated-measures ANOVA: $F_{1, 23} = 1.03$, $P = 0.321$, post-breeding: $F_{1, 21} = 2.652$, $P = 0.1183$, Fig. 1.4). Also, implant type had no effect on bite force in the breeding season or post-breeding season experiments (breeding: $F_{1, 23} = 0.08$, $P = 0.78$, post-breeding: $F_{1, 23} = 2.31$, $P = 0.142$, Fig. 1.4). Bite force did not change over time in the breeding season or post-breeding season experiments (breeding: $F_{2, 22} = 1.67$, $P = 0.210$, post-breeding: $F_{2, 22} = 3.29$, $P = 0.056$, Fig. 1.4).

DISCUSSION

Here, I investigated the role of T in mediating seasonal increases in bite force and endurance in male *A. sexlineata*. In field-active males, plasma T levels and endurance were greater during the breeding season than the post-breeding season, which was consistent with Gowan (2008). Although there was no relationship between lizard size (SVL or mass), plasma T levels, or endurance, a positive relationship existed between bite force and SVL ($P < 0.001$). Gowan (2008) found that male *A. sexlineata* had greater bite force in the breeding season than the post-breeding season. However, in the present study, bite force was not different between field-active males in the breeding season and post-breeding season (Fig. 1.2). Similar methods were used to measure bite force in both studies, but the sample size of Gowan (2008) was much larger than that of the present study. Using a power analysis (Sokal and Rohlf 1995) with my sample size ($N_1 = 27$, $N_2 = 28$) and $\alpha = 0.05$, I had an 80% chance of correctly detecting an effect size of 0.491 N in bite force between seasons whereas Gowan (2008) had the power to detect an effect size of 0.253 N with a sample size of $N_1 = 112$, $N_2 = 33$. Therefore, due to reduced power in the present study, one should use caution when interpreting the bite force data presented here.

My study is the first to show that tonic-release T implants effectively elevate T levels in non-territorial lizards. T-implanted males had elevated T levels for 2 wks during the breeding season and post-breeding season experiments, which was determined through the use of an enzyme immunosorbent assay. Additional support that the T implants elevated T levels was reduced testis volume of T-implanted males in the breeding season experiment (Fig. 1.3). Decreased testis volume was not unexpected considering that exogenous T can reduce testis size through the inhibition of gonadotropin release from the pituitary (Narula et al. 2002, Fusani 2008). In field-active males, testis size naturally decreases during the post-breeding season (Hardy 1962, Etheridge et al. 1986); explaining why empty-implanted males had larger testes during the breeding season experiment than the post-breeding season experiment. Also, because testes were regressed in the post-breeding season, testis volume was not significantly different between treatment groups in the post-breeding season experiment (Fig. 1.3).

Although T implants successfully elevated plasma T levels for 2 wks, they failed to increase bite force and endurance during this time frame. In previous studies involving territorial lizards, exogenous T significantly increased locomotor performance within a short period of time (John-Alder 1994, Klukowski et al 1998, Sinervo et al. 2000). Similar to Sinervo et al. (2000), lizards in the present study were housed in the laboratory throughout the experiment, thereby limiting natural movements (i.e. foraging behavior and mate searching). Because I observed no difference in mass between treatment groups during the breeding or post-breeding season experiments, reduced performance was not due to decreased body condition (Table 1.1). Thus, decreased locomotor performance may be the result of reduced activity levels. Detraining, defined as the “loss of training – induced adaptations, in response to an insufficient training stimulus,” (Mujika and Padilla 2000), may explain the lack of difference in performance between T-implanted and empty-implanted males. If hindlimb muscles atrophied during the experiment, this would explain why most hindlimb muscle masses were not significantly different between treatment groups during the breeding season and post-breeding season experiments (Table 1.2).

The relationship between circulating T and B concentrations varies both within and among species. For example, in males of a single species, the relationship between B and T varies among color morphs (Knapp and Moore 1997) and size classes (Baird and Hews 2007, Husak et al. 2007). Furthermore, when hormone levels are experimentally elevated, T-implanted males have lower B levels compared to controls (Klukowski et al. 1998). Here, I observed a similar trend between exogenous T and plasma B during the post-breeding season experiment (Fig. 1.5). In territorial lizards, subordinate males exhibit increased B levels compared to dominant males (Greenberg et al. 1984). The relationship between B and T following agonistic male-male encounters has yet to be examined in male *A. sexlineata*. However, both of these hormones appear to be important in reproductive behavior because both B and T occur at higher concentrations during the breeding season (Grassman and Hess 1992a).

In a previous lab study involving male *A. sexlineata,* agonistic interactions between males occurred only during the breeding season (Brackin 1978). Although Brackin (1978) did not quantify hormone concentrations, the smaller, less aggressive males likely had elevated B levels, which was later evidenced by increased adrenal volumes and decreased testis size compared to larger males. A study set up similar to Brackin (1978) in which males are ranked (based on dominance) would provide additional information about the roles of B and T in the mating system of *A. sexlineata*.

Although elevated T levels are often associated with agonistic behavior, the purpose of elevated B levels during the breeding season remains unknown for male *A. sexlineata*. During the breeding season, the probability of a male encountering a predator is likely increased due to intensive foraging and mate searching efforts (Anderson and

Vitt 1990). Because B is involved in the stress response of many animals, elevated B levels may increase wariness or sprint speed in the "flight" response of males to predators or conspecifics (Miles et al. 2007). Male *A. sexlineata* are often observed sprinting between bushes or across open sand (i.e. jeep trails, personal observation); therefore, elevated sprint speed may increase survival rates of males if they are better able to escape from predators. While elevated endurance (as a result of increased T, John-Alder 1994) allows territorial males to defend larger territories and increase their display rate (i.e. head bobs and dewlap extensions, Perry et al. 2004), the role of T in mediating seasonal increases in performance of non-territorial species deserves more attention. Elevated endurance levels during the breeding season may be important for male *A. sexlineata* during the breeding season by enabling them to forage for longer periods of time or increase the duration of mate-guarding behavior, both of which could increase reproductive fitness.

Several factors related to captivity may have affected the results seen here, including: lack of agonistic interactions between males, the absence of receptive females, or other environmental cues necessary to induce breeding-season behavior (Moore 1988, Neal and Wade 2007). Future studies examining additional morphological and physiological traits that may be under the influence of T would be beneficial in further isolating the role of T in non-territorial mating systems. Furthermore, a study examining the relationship between circulating hormone levels, population size, and factors related to predator escape (i.e. degree of wariness) would be informative in describing the seasonal increases of locomotor performance that occur in male *A. sexlineata* during the breeding season.

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Table 1.1. Mean ± SE morphological and performance variables of implanted lizards during the breeding season and post-breeding season experiments. Sample sizes are given in parentheses. Endurance decreased significantly for all treatment groups.

	$\bar{x} \pm$ SE Pre-treatment	$\bar{x} \pm$ SE Post-treatment
Breeding season		
T-implant		
SVL (mm)	63.23 ± 1.55 (13)	63.42 ± 1.55 (12)
Mass (g)	6.57 ± 0.49 (13)	6.65 ± 0.45 (12)
Testosterone (ng/mL)	7.26 ± 2.91 (4)	1.44 ± 0.81 (2)
Maximum Bite Force (N)	3.61 ± 0.33 (13)	3.68 ± 0.29 (12)
Maximum Time (s)	230.54 ± 21.16 (13)	171.5 ± 18.63 (12) *
Breeding season		
Empty-implant		
SVL (mm)	63.36 ± 1.81 (14)	62.77 ± 1.41 (13)
Mass (g)	6.39 ± 0.53 (14)	6.47 ± 0.57 (13)
Testosterone (ng/mL)	3.03 ± 1.33 (8)	0.38 ± 0.07 (8)
Maximum Bite Force (N)	4.13 ± 0.44 (14)	3.87 ± 0.30 (13)
Maximum Time (s)	267.86 ± 22.67 (14)	168.46 ± 24.14 (13) *
Post-breeding season		
T-implant		
SVL (mm)	66.31 ± 0.86 (13)	66.08 ± 1.10 (13)
Mass (g)	7.90 ± 0.28 (13)	7.32 ± 0.51 (13)
Testosterone (ng/mL)	1.84 ± 1.06 (5)	0.97 ± 0.40 (5)
Maximum Bite Force (N)	4.60 ± 0.34 (13)	4.01 ± 0.24 (12)
Maximum Time (s)	159.85 ± 15.63 (13)	101.45 ± 8.76 (11) *
Post-breeding season		
Empty-implant		
SVL (mm)	63.36 ± 1.07 (14)	63.46 ± 1.15 (13)
Mass (g)	6.81 ± 0.39 (14)	7.14 ± 0.51 (13)
Testosterone (ng/mL)	0.77 ± 0.24 (6)	0.38 ± 0.24 (2)
Maximum Bite Force (N)	3.40 ± 0.27 (14)	3.87 ± 0.19 (13)
Maximum Time (s)	127.29 ± 10.59 (14)	94.54 ± 5.94 (13) *

Table 1.2. Mean ± SE hindlimb muscle masses (mg) and muscle diameters (mm) of males during the breeding season and post-breeding season experiments. Note that only the diameter of the ambiens pars ventralis was greater in the T-implanted males during the breeding season.

	Breeding Season		Post-breeding Season	
Hindlimb muscle	Control (N = 6)	T (N = 6)	Control (N = 7)	T (N = 4)
iliofibularis (IF)				
Mass	9.78 ± 0.80	13.30 ± 2.08	9.82 ± 1.26	10.28 ± 2.28
Diameter	0.87 ± 0.07	0.78 ± 0.05	0.65 ± 0.09	0.70 ± 0.19
flexor tibialis internus (FTI)				
Mass	3.15 ± 0.26	3.97 ± 0.51	2.98 ± 0.40	3.30 ± 0.53
Diameter	0.38 ± 0.02	0.43 ± 0.02	0.65 ± 0.05	0.33 ± 0.09
pubotibialis (PT)				
Mass	6.48 ± 0.51	8.07 ± 1.27	7.07 ± 1.00	6.80 ± 0.92
Diameter	0.37 ± 0.08	0.40 ± 0.04	0.35 ± 0.02	0.28 ± 0.08
puboischiotibialis (PIT)				
Mass	13.23 ± 1.34	15.62 ± 1.99	13.82 ± 1.83	14.10 ± 2.65
Diameter	0.45 ± 0.07	0.48 ± 0.07	0.38 ± 0.05	0.35 ± 0.09
gastrocnemius pars major (G major)				
Mass	12.58 ± 1.15	17.45 ± 2.22	14.52 ± 1.70	13.53 ± 2.05
Diameter	0.68 ± 0.22	0.72 ± 0.07	0.68 ± 0.09	0.60 ± 0.15
gastrocnemius pars minor (G minor)				
Mass	17.93 ± 1.78	24.12 ± 2.81	19.18 ± 1.39	21.25 ± 3.59
Diameter	0.88 ± 0.04	0.95 ± 0.03	0.95 ± 0.10	0.85 ± 0.16
iliotibialis (IT)				
Mass	8.18 ± 0.61	13.23 ± 1.37	10.47 ± 1.47	10.10 ± 1.96
Diameter	0.57 ± 0.06	0.60 ± 0.05	0.57 ± 0.08	0.45 ± 0.10
ambiens pars ventralis (AmbV)				
Mass	10.37 ± 0.97	13.63 ± 2.14	11.63 ± 1.72	11.10 ± 2.09
Diameter	0.67 ± 0.05*	0.88 ± 0.08	0.77 ± 0.12	0.58 ± 0.08

*P < 0.05, **P < 0.01.

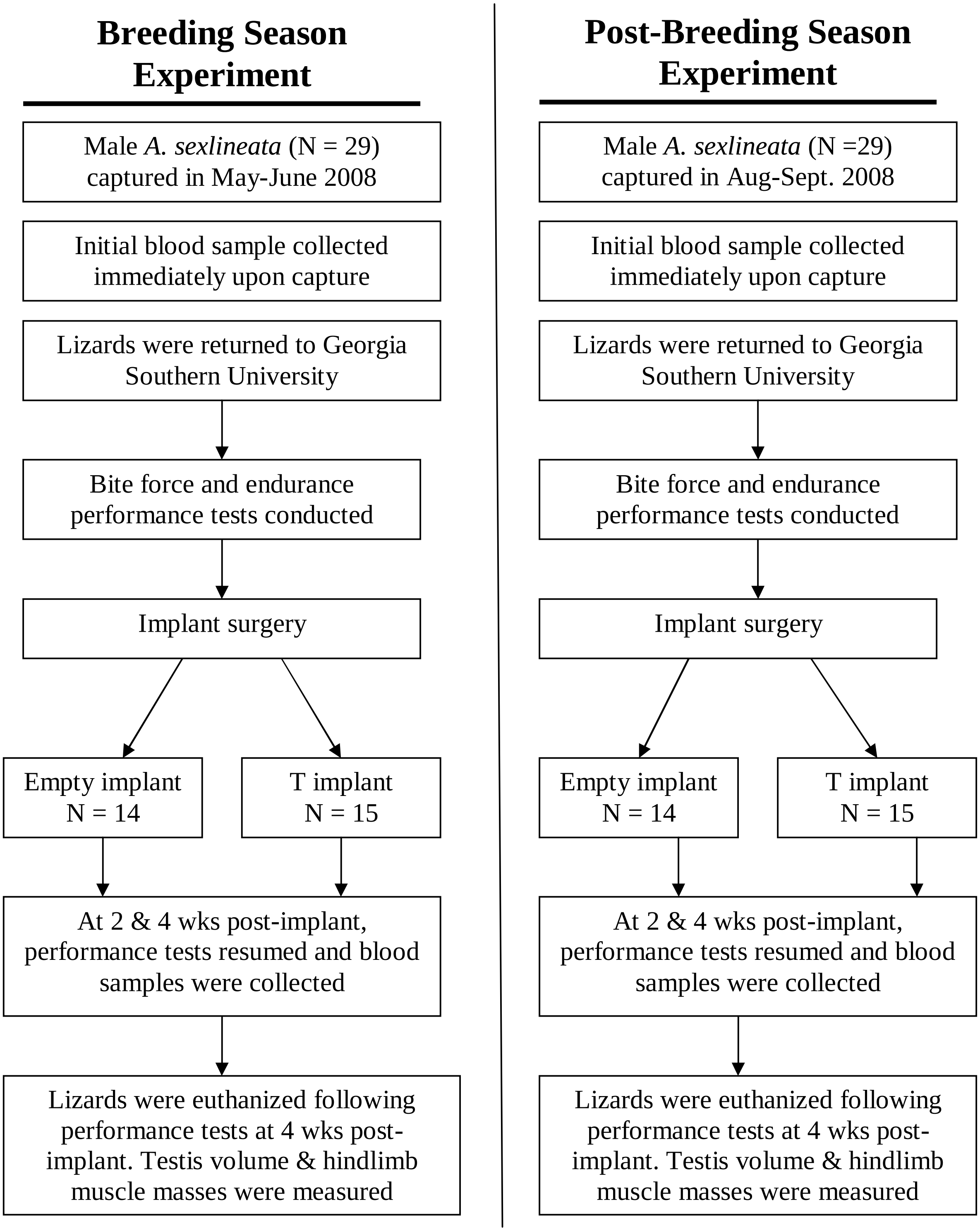


Figure 1.1. Experimental design of the implant study. Twenty-nine male *A. sexlineata* were captured in the breeding season and post-breeding season. Those lizards were randomly divided into 2 groups: T implant or empty implant. Following the implant surgery, lizards were allowed to recover for five days, after which performance testing resumed.

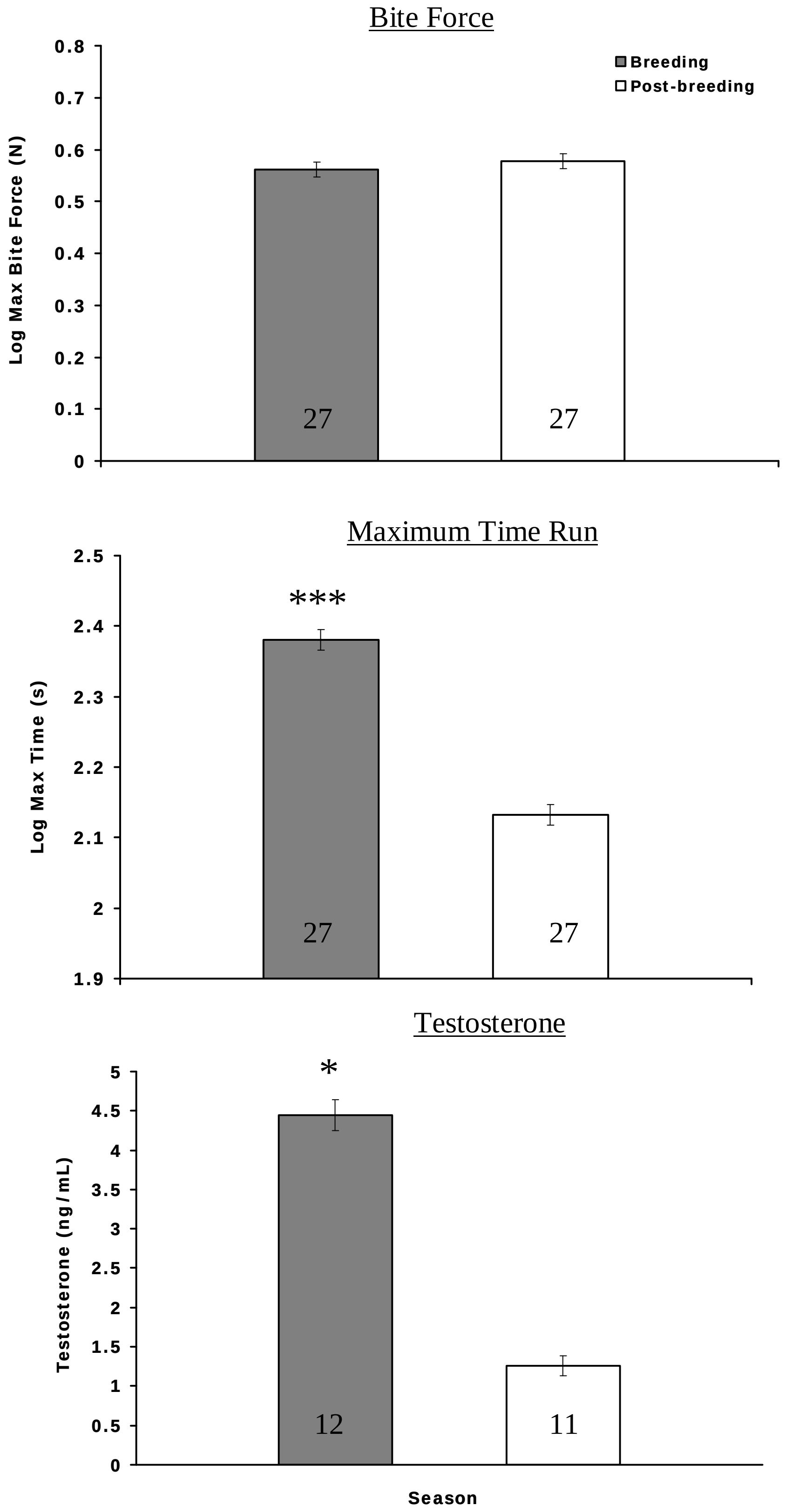
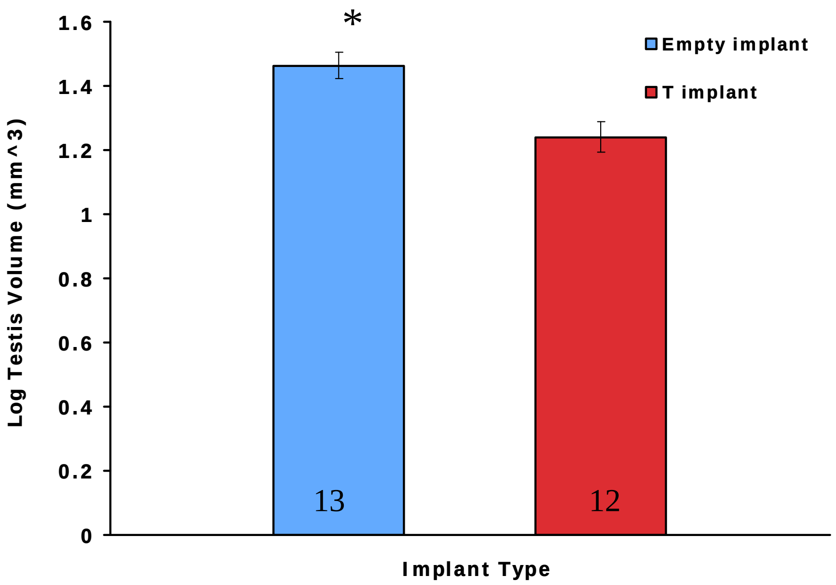


Figure 1.2. Initial values for bite force, maximum time run (s), and testosterone in the breeding and post-breeding season. Data are displayed as mean (± 1 SE). Sample sizes are given at the bottom of each bar.

Breeding Season



Post-breeding Season

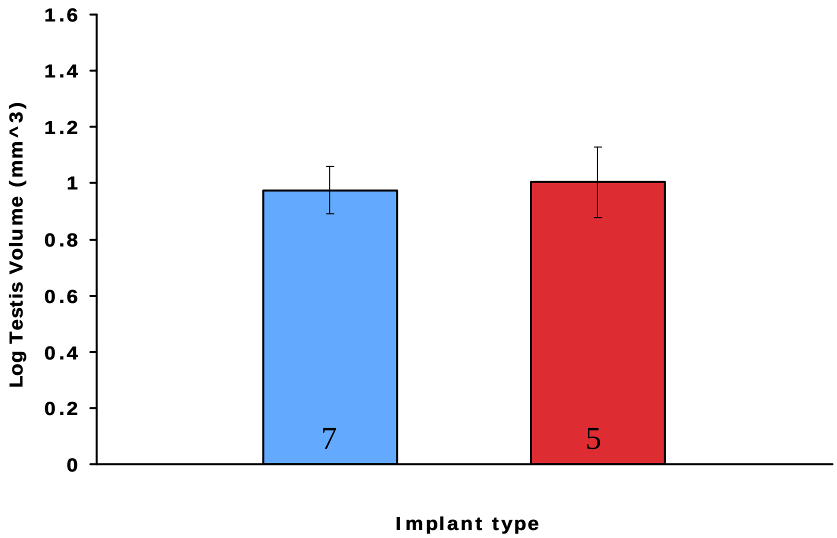


Figure 1.3. Log average testis volume of implanted males during the breeding season and post-breeding season experiments. T-implanted males had smaller testis volume than empty-implanted males in the breeding season experiment. Testis volume was not significantly different between treatment groups in the post-breeding season experiment. Values are presented as mean (± 1 SE). Sample sizes are shown at the bottom of each bar.

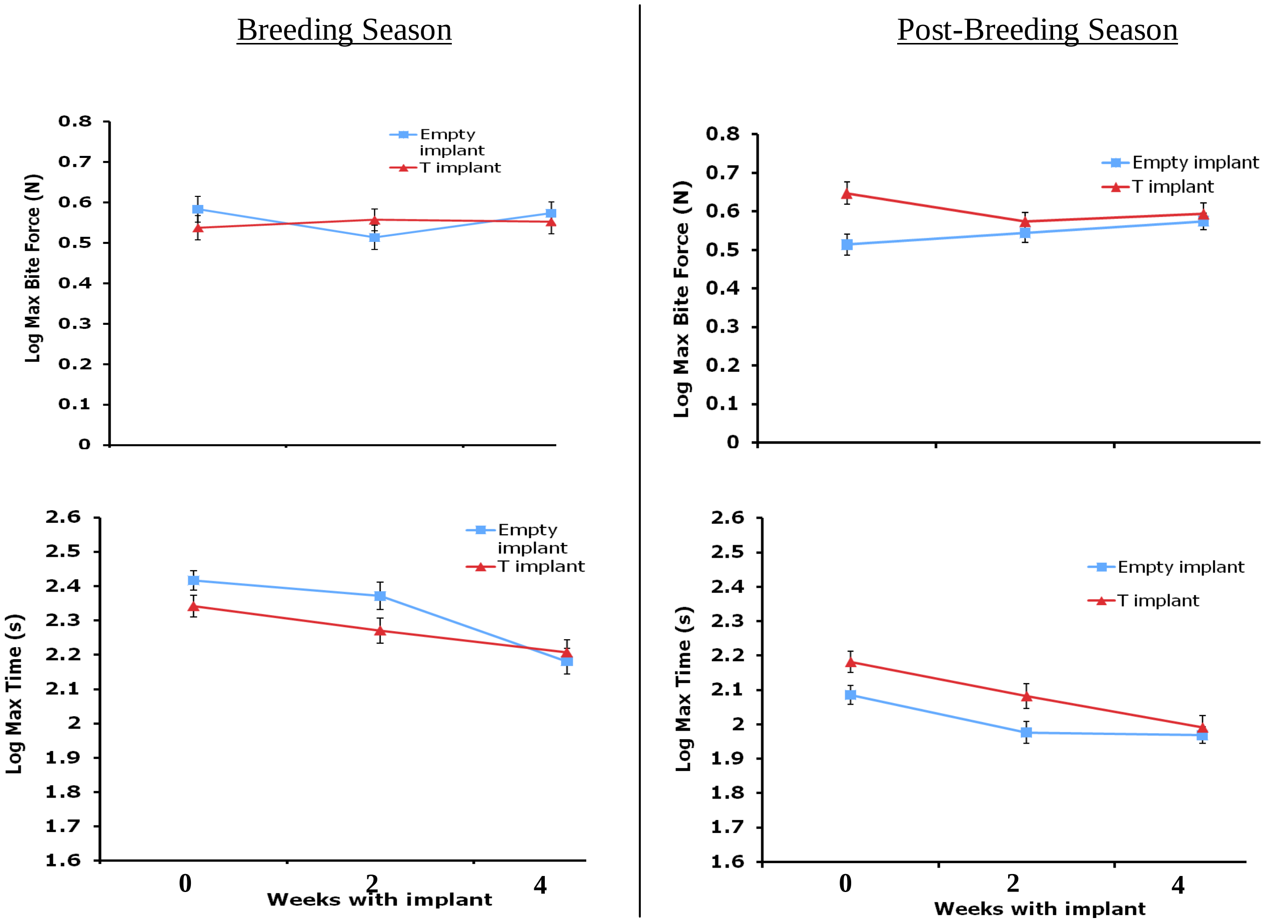


Figure 1.4. Mean (± 1 SE) values for bite force and maximum time run during the breeding season and post-breeding season experiments at 0, 2, & 4 wks post-implant. Max time decreased for all groups over the 4 wk period. Bite force did not change over the 4 wk period for any treatment group. Sample sizes are presented in Table 1.1.

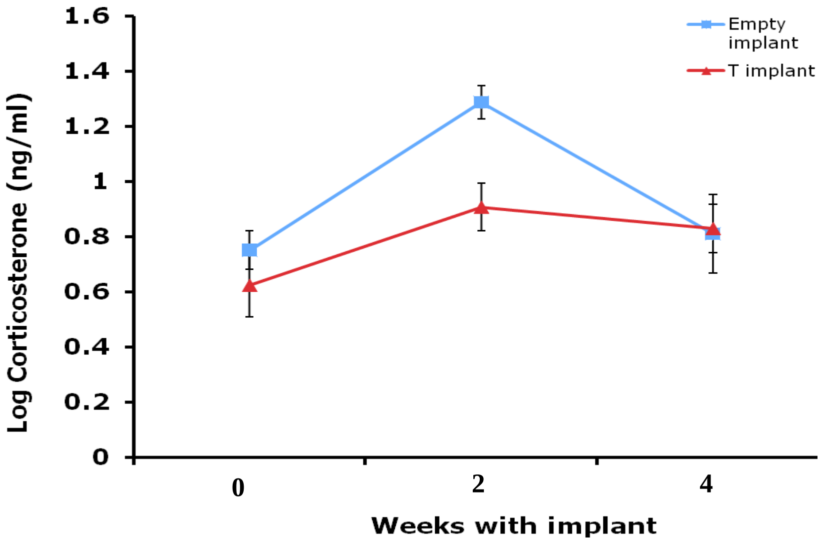


Figure 1.5. Corticosterone concentrations for male *A. sexlineata* implanted (with either an empty or T implant) during the post-breeding season experiment at 0, 2, & 4 wks post-implant. T-implanted males had lower corticosterone concentrations than empty-implanted males at 2 wks post-implant.

CHAPTER 2

EFFECTS OF TRAINING AND TESTOSTERONE ON LOCOMOTOR PERFORMANCE IN MALE SIX-LINED RACERUNNERS (*ASPIDOSCELIS SEXLINEATA*)

ABSTRACT

In territorial lizards, testosterone (T) is known to affect a variety of traits that are important for fitness. Traits influenced by T in territorial males include coloration, locomotor performance, territorial display rate (i.e. head bobs and push-ups), and the size of sexual ornaments (i.e. dewlap). In a previous lab study, experimentally elevated T levels in male *Aspidoscelis sexlineata* did not result in increased performance (i.e. bite force and endurance). Instead, endurance decreased for all lizards, suggesting that captivity likely negatively impacted endurance. Thus, the aim of the current study is to isolate the effect of training on locomotor performance in male *A. sexlineata*. Additionally, lizards were given T-implants to determine if T supplementation could increase locomotor performance more than training alone. Lizards were subjected to a 6-wk training regimen consisting of three performance tests: treadmill endurance, burst distance, and maximum time run. In addition to measuring changes in locomotor performance, ventral coloration, percent hematocrit, and testis volume were measured. Neither training alone nor training in combination with T resulted in increased locomotor performance. Hue and hematocrit decreased for all lizards over the 6-wk training regimen. My results suggest that ventral coloration is mediated by T in male *A. sexlineata* and that an inverse relationship exists between T and corticosterone (a stress hormone) in this species.

INTRODUCTION

In several lizard species, males exhibit seasonal plasticity in performance (Irschick et al. 2006, Garland and Else 1987). Increased performance is often associated with elevated hormone levels (especially testosterone, Gowan 2008, John-Alder 1994, Klukowski et al. 1998, Sinervo et al. 2000). Elevated testosterone (T) levels have been associated with increased dominance and aggression in males (Moore 1988, Sinervo et al. 2000, Tokarz 1987, Tokarz 1995). Furthermore, elevated T levels have been linked to increased display rate (Tokarz et al. 2002), bite force (Irschick et al. 2006), and endurance (Sinervo et al. 2000, John-Alder 1994, John-Alder et al. 2009), all of which may lead to increased fitness.

The role of hormones in mediating seasonal plasticity of performance has been studied extensively in territorial lizards (Garland and Else 1987, Irschick et al. 2006 and references therein). However, the effect of T on performance in non-territorial species has received little attention. In the wide-foraging lizard, *Aspidoscelis sexlineata*, males have elevated T and endurance during the breeding season (Grassman and Hess 1992a, Gowan 2008), which suggests that T may be involved in seasonal increases in endurance. In this species and most non-territorial lizard species, once a receptive female has been located, elevated endurance would allow a male to mate-guard her for longer periods of time. Both increased duration of foraging activity and increased mate-guarding could enhance a male's fitness. Several studies have observed increased performance (i.e. bite force and endurance) as a result of elevated T levels, but determining the morphological or physiological factors responsible for the increased performance has proven difficult.

In a previous study, I showed that tonic-release T implants effectively elevated plasma T levels of male *A. sexlineata* for 2 wks (Ch. 1). Because plasma T levels and performance vary seasonally in field-active male *A. sexlineata* (Gowan 2008, Ch. 1), the present study was designed to further isolate the roles of T in mediating performance. In my previous lab study (Ch. 1), elevated T levels did not result in increased performance. Endurance decreased for all lizards (i.e. T-implanted and empty-implanted males) during the breeding season and post-breeding season experiments, suggesting detraining (an effect of captive housing) as a possible explanation for the observed decrease in performance. Detraining is defined as “the partial or complete loss of training-induced adaptations, in response to an insufficient training stimulus”, Mujika and Padilla (2000).

The effect of training on performance in reptiles has received little attention. In addition to the current study, only three studies to date have examined the effects of training on locomotor performance in lizards (Gleeson 1979, Garland et al. 1987, Jones 2000). Most recently, Jones (2000) examined the effects of endurance training (on a treadmill) and sprint training (around a circular racetrack) on locomotor performance in male and female *Aspidoscelis sexlineata* by exercising the lizards 4 days per week for 6 weeks. Garland et al. (1987) subjected lizards to a training regimen consisting of several 30-min treadmill endurance trials per week, which seemed excessive because half of the lizards in the training group (n = 10) experienced deterioration of hindlimb joints and muscles. Gleeson (1979) ran lizards around a circular track 5 days per week and gradually increased the total distance run (i.e. week 1 = 13m, week 2 = 27 m, etc.). The training regimen of Gleeson (1979) led to reduced sprint speed; possibly as a result of decreased thigh muscle mass. To date, none of the training studies involving lizards have successfully increased locomotor performance as a result of training.

Several studies involving human subjects have examined how T supplementation in addition to training affects muscle properties (i.e. fiber composition and fiber diameter). However, most of these studies have used supraphysiological levels of T (Griggs et al. 1989, Sinha-Hikim et al. 2002) or T in combination with other hormones (Alén et al. 1984). Elevated T levels have been shown to increase muscle mass (Griggs et al. 1989, Huyghe et al. 2009), muscle fiber diameter (Alén et al. 1984, but see Griggs et al. 1989), muscle size (Bhasin et al. 1996), percent hematocrit (Griggs et al. 1989), and strength (Bhasin et al. 1996, Bhasin et al. 2001) in males. Given these findings, my study focuses on answering the following questions: (1) Does exogenous T or training affect percent hematocrit in male *A. sexlineata* during the breeding season? (2) Can a training regimen consisting of sprint training and endurance training increase locomotor performance in male *A. sexlineata*? (3) Does T supplementation in addition to training produce greater increases in performance (i.e. treadmill endurance, burst speed, or maximum time run) than training alone? (4) Does exogenous T affect ventral coloration in male *A. sexlineata*?

Lizards with greater endurance are expected to have higher percent hematocrit than lizards with lower endurance (Garland 1993) due to increased oxygen demands during activity. In male *Sceloporus undulatus*, percent hematocrit, endurance, and T are greatest during the breeding season (John-Alder et al. 2009). Therefore, it is likely that T affects endurance in *A. sexlineata* . I expect that *A. sexlineata* will have high percent hematocrit due to their high endurance and high percent time moving values (Garland

1993, Garland 1999). Because both training (in *Amphibolurus nuchalis*: Garland et al. 1987) and exogenous T (Griggs et al. 1989) have been shown to increase percent hematocrit, I predict that males in the T + training group will exhibit the greatest increase in endurance. Exogenous T affects several morphological traits that may lead to increased locomotor performance including: muscle mass (Griggs et al. 1989), fiber diameter (Alén et al. 1984, but see Griggs et al. 1989), and muscle size (Bhasin et al. 1996). Because the combination of T and training yields greater results than either T or training alone (Bhasin et al. 1996), I predict that males in the T + training group will have the greatest increase in endurance.

In addition to examining how T and training affect locomotor performance in male *A. sexlineata*, I am interested in examining how T affects testis volume and coloration in non-territorial lizards. T mediates variation in coloration of territorial males lizards (*Sceloporus jarrovii*: Cox et al. 2008, *Sceloporus undulatus*: John-Alder et al. 1996); therefore, I predict that T-implanted males will have increased hue compared to empty-implanted males. Furthermore, because exogenous T decreases testis volume (Ch. 1), I expect to find similar results here.

METHODS

Field site and sampling

Male *A. sexlineata* (SVL ≥ 54 mm, Hoddenbach 1966) were captured in Ocala National Forest in April 2009. All lizards were captured within 5 min of being sighted and blood samples (50-100 μL) were collected from the post-orbital sinus (MacLean et al. 1973, Grassman and Hess 1992b) within 3 min of capture using two heparinized capillary tubes. Upon capture, the sex of each lizard was confirmed by everting the hemipenes. Blood samples were centrifuged immediately on site to isolate the plasma fraction and hematocrit (packed red blood cell volume) was measured using dial calipers. For each lizard, mean percent hematocrit was calculated from the two heparanized capillary tubes used to collect the blood sample. All blood samples were stored on dry ice in the field and subsequently stored at -20°C. Lizards were kept in cloth holding bags until they were returned to the animal facility at Georgia Southern University.

Housing

Lizards were housed separately in glass aquaria (dimensions: 50.8 x 27.9 x 33.0 cm^3) with a sand substrate. Cardboard inserts between aquaria obscured sight lines and prevented agonistic interactions between lizards. Each aquarium contained a water dish, hide, and basking spot under an incandescent bulb. Lizards were fed to satiation every other day. The diet consisted of crickets (dusted with Reptocal®) and mealworms. Lighting was controlled by automatic timers on an 11L: 13D light cycle, and UVB was provided daily. All lizards were maintained in the lab for 6 wks.

Performance

Three performance traits were tested: burst distance, maximum time run, and treadmill endurance. Lizards were warmed to their field-active body temperature (38 - 41°C, personal observation) for 30 min prior to each performance trial, and temperatures were verified before and after each trial. All performance tests were conducted between 0900-1400 h. Lizards performed one test per day, either maximum time run, burst speed, or treadmill endurance. Each week, the training regimen consisted of three burst speed trials, two treadmill endurance trials, and one maximum time run trial.

Careful consideration was taken when designing the training regimen, because previous training studies involving lizards have either been too strenuous (resulting in deterioration of joints and muscles of the hindlimbs, Garland et al. 1987) or have not increased performance possibly because the training regimen was not strenuous enough (Gleeson 1979, Jones 2000). Therefore, in the present study, I tried to increase the difficulty of the training regimen implemented by (Jones 2000). Here, lizards were subjecting to a 6 d/wk training regimen consisting of both endurance training and sprint training.

Maximum time run (s) was measured by chasing a lizard around a circular racetrack measuring 5-m in circumference with cardboard walls and artificial turf substrate. Each lizard was chased around the track at full speed until it began to slow. Upon slowing, tail taps were used to motivate the lizard to continue its forward motion. Throughout the trial, the total distance covered by the lizard was recorded every 30 s onto a voice recorder. The trial ended when the lizard failed to move forward after ten consecutive taps on the tail and the lizard had lost its righting response (Cullum 1997, Cullum 1998). If the lizard was able to right itself upon being turned on its back, the trial continued until the lizard was exhausted. Burst distance (m) was measured using the same methods described for maximal time run, except that the trial ended when the lizard stopped sprinting in response to being chased. Treadmill endurance (s) was measured on a small animal treadmill at a constant speed of 1.0 km/hr. Lights suspended above the belt ensured that lizards maintained their field-active temperatures throughout the trial. Lizards were encouraged to run by lightly tapping them on the tail. Minimal stimulation was used to motivate the lizards while on the treadmill because if frightened, the lizards would dart to the front of the treadmill, not maintain a constant speed, and tire easily. The trial ended when the lizard was unable to maintain its position on the belt after ten consecutive taps on the tail (Huey et al. 1984). The longest trial of each performance test (before and after the implant and training regimen) was retained for statistical analysis.

Implants

Following initial performance trials, lizards were randomly assigned to one of three treatment groups: T implant + training (T + Training), empty implant + training (∅ + Training), or empty implant without training (∅ ∅). Silastic® tubing (Dow Corning, Cat. No. 508-006, 1.47 mm ID x 1.96 mm OD) measuring 4-mm in length was used to construct the implants. One end of the implant was sealed with silicone sealant and cured for approximately 2 h. Then, the control (empty) implants were filled with 3 μL of dimethyl sulfoxide (DMSO), and the open end of the tube was closed with silicone sealant. Using a Hamilton® syringe, T implants were injected with 3 μL of a solution containing T (Sigma, T-1500) dissolved in DMSO at a concentration of 100 μg T/μL of DMSO (Cox et al. 2005). Then, the open end of the implant was sealed with silicone sealant. All implants were placed under a fume hood for several days to cure.

Before the implant surgery, lizards were anesthetized with ketamine injected intramuscularly. For each implant, a small slit was made in the right lateral fold of the lizard (slightly anterior to the hindlimb). The implant was inserted subcutaneously and the incision site was sealed with 3M Vetbond tissue adhesive. Lizards were held in cloth bags for at least 1 h post-surgery to allow the adhesive to set. Lizards were allowed to recover from surgery for 5 d before performance trials resumed.

Color

The ventral surface of each lizard was scanned every 2 wks using an HP Photosmart C4280 all-in-one scanner. Lizards were placed in cloth bags, briefly cooled to reduce struggling, and then their ventral surface was scanned. Images were saved onto a laptop and color was analyzed using Adobe PhotoShop (version 6.0) following the methods described by Cox et al. (2008). The "elliptical marquee" tool was used to select three areas across the width of lizard's ventral surface. The total area selected covered a majority of the lizard's body width. For each ellipse, the histogram tool was used to quantify the mean red, green, and blue values. Then, the color picker tool was used to quantify the hue, saturation, and brightness. The mean value of hue, saturation, and brightness for each lizard was calculated and retained for statistical analyses.

Analysis

All statistical analyses were performed using JMP ® (version 7.0.1). Significance was accepted at $P < 0.05$ and all P values are two-tailed. Testis volume and body mass were $\log_{10}$ transformed due to non-normal distributions. A Kruskal-Wallis test was used to compare treatment groups when data could not be transformed to meet the parametric assumptions. A repeated-measures ANOVA was used to analyze differences in ventral coloration, percent hematocrit, and performance variables over the 6-wk period. Sample sizes differ for performance tests and testis volume because a subset of lizards was sent to Clemson University for histochemical analysis of hindlimb muscles as part of a future study. Therefore, testis volume was not measured on these lizards. Additionally, due to time constraints when capturing lizards in the field, initial percent hematocrit was not measured on all lizards ($n_{\varnothing + \text{Training}} = 7$, $n_{T + \text{Training}} = 7$, $n_{\varnothing \varnothing} = 6$).

RESULTS

Treatment groups did not differ in initial SVL (Kruskal-Wallis, H = 0.03, P = 0.983, $n_{\varnothing + \text{Training}} = 10$, $n_{\text{T + Training}} = 9$, $n_{\varnothing \varnothing} = 10$), mass (Kruskal-Wallis, H = 0.04, P = 0.982), or percent hematocrit (ANOVA, $F_{2, 16} = 3.06$, P = 0.075, Fig. 2.1). Training significantly reduced body mass (repeated-measures ANOVA, $F_{1,18} = 12.15$, P = 0.003). Males in the T + Training and ∅ + Training groups decreased in mass ($F_{1,17} = 11.94$, P = 0.003), while males in the ∅ ∅ group increased in mass ($F_{1,9} = 9.19$, P = 0.014). There was no effect of T supplementation on mass (repeated-measures ANOVA, $F_{1, 17} = 0.018$, P = 0.915).

Although percent hematocrit decreased over time for all males (repeated-measures ANOVA, T + Training: $F_{2,4} = 16.70$, P = 0.003, ∅ + Training: $F_{2,3} = 13.12$, P = 0.033, ∅ ∅: $F_{2,4} = 24.48$, P = 0.006) there was a significant effect of T on percent hematocrit ($F_{1,9} = 1.86$, P = 0.003). Males in the T + Training group had greater percent hematocrit than males in the ∅ + Training group over the 6-wk training regimen. Percent hematocrit was not affected by training ($F_{1,9} = 2.78$, P = 0.130).

Color

Pre-treatment values of ventral coloration (i.e. hue, saturation, and brightness) were not different among treatment groups (Kruskal-Wallis, hue: H = 0.15, P = 0.927, saturation: H = 1.98, P = 0.327, brightness: H = 3.54, P = 0.170). T supplementation did not affect ventral coloration (repeated-measures ANOVA, hue: $F_{1,15} = 0.18$, P = 0.677, saturation: $F_{1,15} = 0.03$, P = 0.871, brightness: $F_{1, 15} = 1.99$, P = 0.179). Also, training had no effect on ventral coloration (hue: $F_{1, 16} = 0.04$, P = 0.848, saturation: $F_{1, 16} = 1.15$, P = 0.299, brightness: $F_{1, 16} = 0.693$, P = 0.417). Hue decreased significantly over time for males in the ∅ ∅ and T + Training groups, but not the ∅ + Training group (repeated-measures ANOVA, ∅ + Training: $F_{2, 6} = 4.35$, $P = 0.068$, T + Training: $F_{2,7} = 129.59$, $P < 0.001$, ∅ ∅: $F_{2,8} = 10.97$, $P = 0.005$, Table 2.1). No change in saturation or brightness occurred over time for any treatment group.

Performance

Body mass explained a significant amount of variation in pre-treatment maximum burst distance ($r^2 = 0.248$, $P = 0.030$); therefore, residual values were used in analyses. Pre-treatment performance values were not different among sampling sites within Ocala National Forest (ANOVA, maximum burst distance: $F_{3, 25} = 1.39$, $P = 0.268$, maximum treadmill endurance: $F_{3,14} = 1.39$, $P = 0.287$, maximum time run: $F_{3, 25} = 0.13$, $P = 0.940$). Furthermore, pre-treatment performance was not different between the training groups (ANOVA, maximum burst distance: $F_{1, 17} =2.10$, $P = 0.1433$, maximum time run: $F_{1, 17} = 0.82$, $P = 0.378$; maximum treadmill endurance (s): $F_{1, 17} = 0.08$, $P = 0.779$). T supplementation did not increase locomotor performance more than training alone (repeated-measures ANOVA, maximum burst distance: $F_{1,15} = 0.10$, $P = 0.754$, maximum time run: $F_{1,15} = 0.62$, $P = 0.444$, maximum treadmill endurance: $F_{1,14} = 0.52$, $P = 0.481$). Also, locomotor performance did not change over time as a result of training (repeated-measures ANOVA, maximum burst distance: $F_{1,15} = 0.20$, $P = 0.658$, maximum time run: $F_{1,15} = 2.16$, $P = 0.162$, maximum treadmill endurance: $F_{1,14} = 1.69$, $P = 0.215$).

Testis Volume

Testis volume was significantly different among the three treatment groups (ANOVA, $F_{2,10} = 8.06$, $P = 0.008$). Males in the T + Training and ∅ + Training groups had smaller testis volume than males in the ∅ ∅ group. Testis volume of lizards in the lab study was compared to that of field-active males ($n = 5$) captured on May 17, 2009 in Ocala National Forest (at the same study site) to determine if captivity reduced breeding season condition. Testis volume of field-active males was greater than that of T + Training and ∅ + Training males; however, testis volume of field-active males and ∅ ∅ males was not significantly different (ANOVA, $F_{3, 14} = 12.69$, $P < 0.001$, Tukey-Kramer HSD, Fig. 2.2).

DISCUSSION

Here, I attempted to isolate the effects of training and testosterone supplementation on locomotor performance in male *A. sexlineata*. Lizards were subjected to a 6-wk training regimen, which incorporated both sprint training and endurance training. The training regimen did not seem overly strenuous because there were no signs of injury to indicate that the training regimen was excessive such as in Garland et al. (1987). Although body mass decreased over time for males in the training groups ($P = 0.003$), all males appeared to be in good health. Despite this, I failed to detect an increase in locomotor performance (Fig. 2.1) thus my results agree with those of earlier training studies involving lizards (Gleeson 1979, Garland et al. 1987, Jones 2000). In other non-human subjects, training regimens lasting several weeks in duration have increased locomotor performance (Cummings 1979, Miller and Camilliere 1981, Pearson et al. 1990, Anttila et al. 2006). In fish, sprint training increases acceleration, burst stamina, and recovery time from exercise (Pearson et al. 1990). Furthermore, endurance training in fish causes increased endurance and increased activity of oxidative enzymes such as succinic dehyrdorgenase in swimming muscles (Anttila et al 2006). In amphibians, both endurance training and sprint training improve locomotor performance over a period of several weeks (Miller and Camilliere 1981). Here, no improvements in the locomotor performance of male *A. sexlineata* occurred following 6 wks of endurance training and sprint training.

Training studies involving humans have found that training in combination with T produces greater increases in performance than T or training alone (Bhasin et al. 1996). Here, T supplementation in addition to training did not increase locomotor performance in male *A. sexlineata*. Because exogenous T and training increase muscle mass and strength (Griggs et al. 1989, Bhasin et al. 1996, Bhasin et al. 2001, Huyghe et al. 2009), it is possible that changes in muscle properties occurred, but were not detected by the performance tests used here. Another explanation for the results seen here is that although T levels were elevated, a supraphysiologic dose of T may be necessary to increase locomotor performance.

Several factors related to captivity may have decreased breeding season condition and thus performance levels in males. Because males were housed in isolation for the duration of the experiment, several environmental cues that may be required to induce breeding season condition (i.e. increased testis volume) were absent. For example, a lack of agonistic interactions among conspecifics and the absence of receptive females may have decreased hue and testis volume. Because field-active male *A. sexlineata* exhibit darker ventral coloration in the breeding season compared to the post-breeding season (Gibbons et al. 2009), I expected to see an increase in hue for males in the T + Training group. However, all treatment groups (∅ ∅, ∅ + Training, T + Training) exhibited a decrease in the hue after 4 wks in captivity (Table 1.2). Similar results were found by

Cox et al. (2008) following castration in male *Sceloporus jarrovii*. Thus, captivity reduces the breeding season condition of males and T influences ventral coloration in this species as well.

In addition to captive housing, training may have reduced breeding season condition of males. If the training regimen was stressful to the lizards, elevated levels of corticosterone (B), a stress hormone, may have suppressed T levels and reduced testis volume (Paolucci et al. 1990, Tokarz 1987). Although exogenous T is known to decrease testis volume (Narula et al. 2002, Fusani 2008); here, it appears that decreased testis volume was the result of increased stress levels. Males in the training groups (T + Training, ∅ + Training) had significantly smaller testis volume than field-active males (Fig. 2.2). Further evidence suggesting that training was stressful to the lizards was indicated by males in the ∅ + Training group having significantly smaller testis volume than males in the ∅ ∅ group (Fig. 2.2). In territorial lizards, *Sceloporus undulatus hyacinthus*, T-implanted males have lower B levels compared to controls (Klukowski et al. 1998). Thus, it is likely that an inverse relationship exists between B and T in male *A. sexlineata* because this would explain why males in the T + Training group had greater testis volume compared to males in the ∅ + Training group (Fig. 2.2).

The effect of elevated B levels on percent hematocrit varies across lizard species. Elevated B has been shown to either increase percent hematocrit (Berger et al. 2005) or have no effect (Miles et al. 2007, Mills et al. 2008). Here, I expected to see the greatest increase in percent hematocrit in T + Training males because both training and elevated androgen levels have been shown to increase percent hematocrit (Alén 1985, Garland et al. 1987). Although percent hematocrit decreased over time for all treatment groups (P <

0.04), T supplementation resulted in greater percent hematocrit (P = 0.003, Table 2.1). In several species, hematocrit varies seasonally in males. For example, in male iguanas, hematocrit is lower in the breeding season compared to the post-breeding season (Acuña 1974). Without knowing the seasonal profile of hematocrit in male *A. sexlineata*, it is difficult to determine if decreased hematocrit was a result of increased stress levels, training, or natural seasonal variation.

In addition to reducing reproductive condition in males, increased corticoid levels may negatively impact locomotor performance. If muscle properties of lizards are affected by increased stress levels, as seen in mammals (Fimbel et al. 1993), this could have detrimental effects on locomotor performance. Therefore, histochemical analysis of the hindlimb muscles of the lizards used in the present study would provide valuable information concerning the effects of elevated T, training, and stress on muscle properties (in progress).

For many lizard species, locomotor performance varies seasonally in males, with the greatest performance capacity occurring during the breeding season (Gowan 2008, Klukowski et al. 1998). In non-territorial lizards, elevated locomotor performance during the breeding season could increase reproductive fitness through increasing the amount of time that a male can search for females as well as lengthen mate-guarding efforts once a receptive female has been located. My results suggest that elevated stress levels may reduce the reproductive condition of males by decreasing testis volume and the hue of ventral coloration. Additional studies on the role of hormones in the locomotor performance of non-territorial lizards would provide valuable insight concerning the underlying morphological (i.e. muscle properties) and physiological factors (i.e. hematocrit) affected by T in non-territorial species.

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Table 2.1 Initial and final values ($\bar{x}$ ± SE) of SVL, mass, and performance for all treatment groups (T implant + training, empty implant + training (∅ + Training), or empty implant without training (∅ ∅). No differences were observed between the pre- and post-treatment values for any treatment group. Sample sizes are given in parentheses.

	$\bar{x}$ ± SE Pre-treatment	$\bar{x}$ ± SE Post-treatment
T implant + Training		
SVL (mm)	62.67 ± 1.94 (9)	62.67 ± 1.93 (9)
Mass (g)	6.99 ± 0.62 (9)	6.20 ± 0.43 (9)
Maximum burst distance (m)	43.44 ± 1.86 (9)	40.89 ± 2.24 (9)
Maximum treadmill endurance (s)	217.37 ± 21.89 (8)	261.33 ± 34.42 (9)
Maximum Time (s)	88.78 ± 18.22 (9)	76.44 ± 19.91 (9)
∅ + Training		
SVL (mm)	63.05 ± 1.83 (10)	62.6 ± 1.82 (10)
Mass (g)	7.02 ± 0.56 (10)	6.01 ± 0.61 (10)
Maximum burst distance (m)	39.7 ± 3.74 (10)	41.50 ± 4.73 (8)
Maximum treadmill endurance (s)	204.5 ± 36.11 (10)	212.0 ± 30.49 (8)
Maximum Time (s)	103.7 ± 16.74 (10)	85.88 ± 10.5 (8)
∅ ∅		
SVL (mm)	62.65 ± 1.80 (10)	62.9 ± 1.82 (10)
Mass (g)	6.95 ± 0.58 (10)	7.69 ± 0.60 (10)
Maximum burst distance (m)	36.2 ± 2.29 (10)	-----
Maximum treadmill endurance (s)	-----	-----
Maximum Time (s)	-----	-----

Table 2.2. Initial and final values ($\bar{x}$ ± SE) of hematocrit and ventral coloration (reported as hue, saturation, and brightness) for lizards in each treatment group. Repeated-measures ANOVA was used to compare pre- and post-treatment values. Hematocrit decreased significantly for all males. Hue decreased significantly for males in the T + Training and ∅ ∅ groups.

	$\bar{x}$ ± SE Pre-treatment	$\bar{x}$ ± SE Post-treatment
T + Training		
Hematocrit (%)	45.6 ± 2.0	32.3 ± 0.9*
Hue	266.1 ± 16.3	188.2 ± 4.7*
Saturation	22.9 ± 3.6	23.3 ± 2.6
Brightness	75.9 ± 2.9	77.8 ± 1.6
∅ + Training		
Hematocrit (%)	40.2 ± 0.9	30.7 ± 3.6
Hue	249.5 ± 28.8	178.9 ± 10.3**
Saturation	24.3 ± 5.0	21.1 ± 3.6
Brightness	81.8 ± 5.1	79.2 ± 5.7
∅∅		
Hematocrit (%)	42.4 ± 1.8	34.3 ± 0.9*
Hue	249.3 ± 20.0	168.6 ± 12.8**
Saturation	17.5 ± 3.8	15.3 ± 2.1
Brightness	76.8 ± 2.1	77.6 ± 2.1

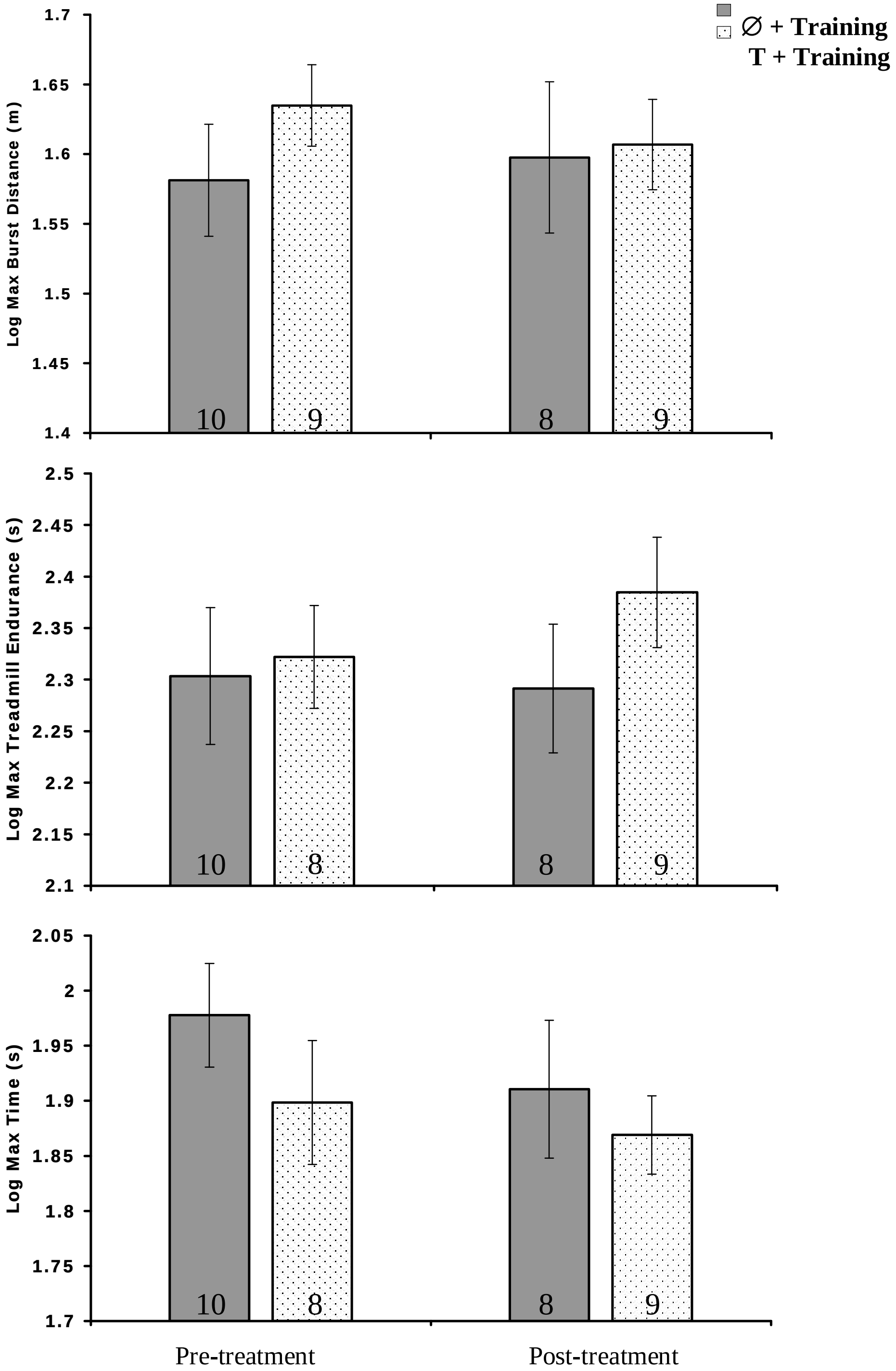

Figure 2.1. Pre- and post-treatment values of locomotor performance tests for males in the ∅ + Training and T + Training groups. Data are presented as mean (± 1 SE). Sample sizes are given at the bottom of each bar.

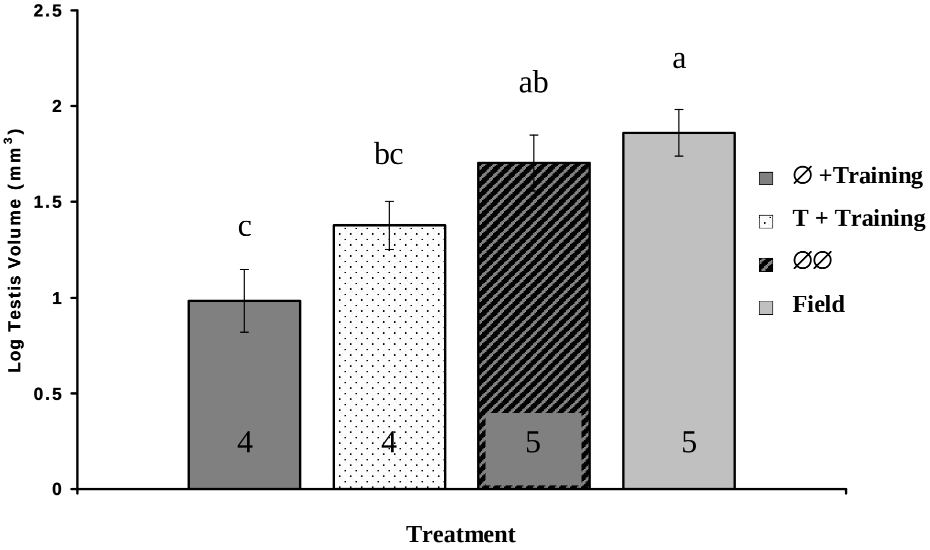

Figure 2.2. Log transformed testis volume of lizards in each treatment. Field-active males were captured on May 17, 2009. Data are presented as mean (± 1 SE). Testis volume was significantly different among groups (ANOVA, $F_{3, 14} = 12.69$, $P < 0.001$). Columns not sharing the same letter are significantly different (Tukey-Kramer HSD).