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Seasonal Patterns of Plasma Testosterone Levels, Bite Force, and Locomotor Performance in Non-Territorial Male Lizards, *Aspidoscelis Sexlineata*

Timothy Adam Gowan

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SEASONAL PATTERNS OF PLASMA TESTOSTERONE LEVELS, BITE FORCE,
AND LOCOMOTOR PERFORMANCE IN NON-TERRITORIAL MALE LIZARDS,

ASPIDOSCELIS SEXLINEATA

by

TIMOTHY A. GOWAN

(Under the Direction of Lance D. McBrayer)

ABSTRACT

The ability to perform key behaviors associated with reproduction, such as biting and locomotion in male lizards, has been linked to social dominance and reproductive success. The underlying mechanisms that govern variation in performance capacity, however, remain unclear. The steroid hormone testosterone mediates numerous traits associated with reproduction in male vertebrates and has been hypothesized to mediate variation in performance. This study examined seasonal patterns of circulating testosterone, morphology, bite force, and locomotor performance in a non-territorial lizard species to address this hypothesis. Male *Aspidoscelis sexlineata* (n=133) were collected throughout the active season, and testosterone levels and performance capacities were measured. Performance capacities were greatest during the breeding season when testosterone levels were elevated. The results of this study support the hypothesis that testosterone levels are related to variation in performance and suggest that seasonal changes in testosterone and performance are timed in a way to maximize reproductive success.

INDEX WORDS: *Aspidoscelis sexlineata*, Testosterone, Bite force, Locomotor performance, Non-territorial, Lizards, Seasonal

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B.S., Towson University, 2005

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CHAPTER 1

INTRODUCTION

Many temperate species are seasonal breeders with reproduction and its associated behaviors restricted to one season. Physiological changes are often linked to this seasonal variation in behavior (Crews and Moore 1986). Specifically, circulating levels of testosterone (T) in males vary seasonally in many temperate vertebrate species and are typically highest during the breeding season when males exhibit sexual behavior (Hau 2007, Wingfield *et al.* 1990 and references therein). Sexual behaviors exhibited by males may include interactions with reproductive females, such as courtship and mating, or with competing males, such as aggressively defending territories or mates. Numerous studies have shown an association between elevated T levels and reproductive behavior and condition (Balthazart 1983, McKinney and Marion 1985, Saino and Moller 1995). While elevated T levels have been linked to an increased expression of behaviors associated with reproduction, less is known regarding whether or not T levels are able to influence the performance capacity of these same behaviors.

Variation among individuals in performance capacities, or the ability to perform ecologically relevant behaviors, is often associated with variation in fitness (Garland and Losos 1994). Thus, studying whole-organism performance traits is useful in understanding correlates of fitness, as selection pressures are predicted to operate directly on the performance capacity of behaviors (Arnold 1983, Husak *et al.* 2006a, Irschick and Meyers 2007). Several studies have shown that the performance capacities of key behaviors used during the breeding season are determinates of reproductive success in male vertebrates, suggesting sexual selection acts on whole-organism performance. For

example, calling properties of birds and anurans, as well as bite force and locomotor performance in lizards, have been linked to social dominance, access to females, and mating success in polygynous species (Ryan and Keddy-Hector 1992, Prohl 2003, Lappin and Husak, 2005, Huyghe *et al.* 2005, Husak *et al.* 2006a,b, Lailvaux *et al.* 2004, Robson and Miles 2000, Perry *et al.* 2004). These findings are not entirely unexpected as these behaviors are used extensively in these species during social interactions, particularly when competing for and courting mates. Although these previous studies demonstrate that the ability to perform certain behaviors are important in determining reproductive output, the underlying mechanisms that fully explain variation in performance capacity are not completely understood. For example, the size and shape of key morphological structures have been correlated with performance of some of these behaviors (Miles 1994, Garland and Losos 1994), however physiological mechanisms such as hormones or enzyme activity may also contribute to variation in performance abilities.

The steroid hormone T has been shown to mediate numerous reproductive traits in vertebrates due to its pleiotropic effects on behavior, morphology, and physiology (see review in Hau 2007). Testosterone has been demonstrated to be an underlying signal controlling a wide spectrum of traits, including courtship, aggression, secondary sexual characteristics, metabolic rate, immune response, and spermatogenesis (Winkler and Wade 1998, Lynch and Blackburn 1995, Buchanan *et al.* 2001, Olsson *et al.* 2000, Hau 2007). Testosterone is also noted for its anabolic properties in the development of skeletal and muscular tissue (Griggs *et al.* 1989, Bhasin *et al.* 1997, Cox and John-Alder 2005). More specifically, androgens such as T can influence the size and physiological properties of muscles associated with male-specific sexual behaviors. Increased

androgen levels have been shown to increase the size of vocal muscles used for advertisement calls in fish and anurans (Brantley *et al.* 1993, Emerson *et al.* 1999), to increase the size of copulatory muscles and motor-neurons in lizards and rodents (Holmes and Wade 2004, Balice-Gordon *et al.* 1990), and to influence the contractile speed of muscles used in calling anurans (Girgenrath and Marsh 2003). Additionally, variation in morphology is predicted to be correlated with variation in performance (Miles 1994, Garland and Losos 1994, Miles *et al.* 2007). Thus, if T levels mediate morphological changes, which in turn influence performance, then variation in T levels may consequently be related to performance capacities and, subsequently, overall fitness. Testing this hypothesis will provide insight on the physiological mechanisms responsible for determining variation in the ability to perform reproductive behaviors and variation in reproductive success.

There has been much recent work examining the variation and correlates of hormones, morphology, performance, and reproductive success in lizards. The results of these studies provide evidence as to which traits are important in determining reproductive success as well as which traits are potentially mediated by T in polygynous lizard species. Social dominance and success in agonistic male-male interactions is related to increased body size and head size (Molina-Borja *et al.* 1998, Perry *et al.* 2004, Huyghe *et al.* 2005). Biting is a behavior often used by male lizards in agonistic contests over resources and during copulation with females, and male lizards with higher bite force are more dominant and have increased mating success (Lailvaux *et al.* 2004, Lappin and Husak 2005, Huyghe *et al.* 2005, Husak *et al.* 2006b). Locomotor performance, as measured by running speed and stamina which are important for agonistic contests

between males and searching for and defending mates, has been shown to predict social dominance, home range size, and reproductive output (Robson and Miles 2000, Perry *et al.* 2004, Husak *et al.* 2006a). As morphology often covaries with performance, key morphological traits, such as head size, head shape, and size of jaw musculature (which determine bite force; Herrel *et al.* 2001, 2007, Verwaijen *et al.* 2002), in addition to the size and shape of limbs (which have been correlated to locomotor performance; Miles 1994, Miles *et al.* 2007) may also be related to social dominance and reproductive success. This study examined seasonal variation in both morphological and performance traits as they relate to seasonal changes in T levels in order to determine the potential of this steroid hormone to influence reproductive success.

Because T is classified as an anabolic steroid that promotes skeletal and tissue development (Griggs *et al.* 1987, Thorarensen *et al.* 1996, Bhasin *et al.* 1997, Cox and John-Alder 2005), it is plausible to predict that elevated T levels can increase the size of structures, such as head size, jaw muscle mass (as suggested by Vitt and Cooper 1985), and limb size, particularly if these morphological structures influence the performance abilities of behaviors associated with reproduction (eg. Biting and locomotion in polygynous lizards). Additional comparisons of physiological and behavioral performance between sexes, morphs, and seasons have also generated the hypothesis that T mediates performance capacity. Males of the lizard genus *Aspidoscelis* (= *Cnemidophorus*) have increased metabolic rates and performed locomotor tasks at greater capacities than females (Cullum 1998). In polygynous lizard species, males also tend to have higher bite force compared to females (Herrel *et al.* 2001, 2007, McBrayer and Anderson 2008). These results suggest that differences in androgen levels might be

at least partially responsible for these differences in performance between sexes. In *Uta stansburiana*, a lizard species comprised of three distinct morphotypes, Sinervo *et al.* (2000) found one morph has higher plasma T levels, endurance, and home range size than the other two morphs, suggesting a possible link between these traits. Furthermore, differences in head size, bite force, and locomotor performance have been documented when comparing lizards sampled from the breeding season to those from the post-breeding season, and these differences may be related to seasonal differences in hormone levels (Cooper and Vitt 1985, Irschick and Meyers 2007, Garland and Else 1987, John-Alder 1984). This hypothesis, however, has not been directly addressed in a study that simultaneously compares seasonal patterns in T levels to seasonal patterns in morphology and performance.

Both manipulative and correlative studies involving territorial Iguanian lizards have revealed a possible link between increased T levels and increased performance capacity. For the lizard species *Anolis sagrei*, *Sceloporus undulatus*, and *Uta stansburiana*, individuals implanted with exogenous T had increased locomotor performance relative to control and castrated individuals (John-Alder 1994, Klukowski *et al.* 1998, Sinervo *et al.* 2000). Additionally, studies that have examined differences in endogenous plasma T levels and performance among morphs within a population reveal that the morphs with higher T levels also have greater endurance and bite force capacity (Sinervo *et al.* 2000, Husak *et al.* 2007). While these studies are valuable in demonstrating a relationship between T levels and performance, few studies have examined naturally occurring hormone levels and how they are related to performance variation; this is especially true for species outside the Iguanian clade with different

evolutionary histories, life history traits, and selection pressures. Additional work on Scleroglossan lizards is thus beneficial in determining if the relationship between T levels and performance that exists in territorial Iguanian lizards is evolutionarily conserved for multiple lineages and reproductive strategies.

In contrast to territorial Iguanian lizards, many Scleroglossan lizards are non-territorial, often do not possess exaggerated sexually dimorphic sexual structures or color badges, and display behavior during aggressive interactions between males is limited or non-existent (Fitch 1958, Carpenter 1962). Without the use of signals and display behavior to predetermine dominance, escalated fights that involve chasing and biting may be more frequent and/or important in species that do not display (Jenssen *et al.* 1995). Also, encounters with novel, competing males may be more frequent in non-territorial species where male home ranges overlap (Marco and Perez-Mellado 1999). Therefore, in contrast to the few studies investigating the relationship between T and the performance of behaviors related to mating in territorial Iguanian lizards, this study used *Aspidoscelis sexlineata*, a non-territorial Scleroglossan lizard, as a model species to examine the relationship between T levels and performance for species with different life histories and mating systems.

Aspidoscelis sexlineata is a terrestrial species that occurs across a large geographic range of the southeastern United States (Carpenter 1960). Members of the lizard family Teiidae, including *A. sexlineata*, are classified as wide-ranging, active foragers with extensive home ranges (Clark 1976, Anderson 1986, Anderson and Vitt 1990, Garland 1993). These large home ranges often overlap with the home ranges of both male and female conspecifics. Because these home ranges are not actively

defended, elaborate display behaviors are not used during social interactions, and individuals have limited fidelity to a specific area, *A. sexlineata* is considered non-territorial (Fitch 1958, Carpenter 1959,1960, Leuck 1985). While these lizards are not known to maintain and defend territories, aggressive intraspecific encounters are nevertheless frequent among males, particularly when competing for access to resources such as receptive females during the breeding season (Carpenter 1960, Leuck 1985). Rather than defending territories, social hierarchies are established by males through aggressive interactions, and multiple males may occupy a given spatial area (Carpenter 1962). Additionally, male *A. sexlineata* have been documented to perform behavior referred to as mate guarding or female tending, where one male will follow and actively defend a female from other males in order to ensure mating opportunities (Carpenter 1962, Leuck 1985, Anderson and Vitt 1990). During these aggressive interactions between males, one male will initially give chase to another. The male being chased may flee in an attempt to escape, demonstrate submission by lying flat on the ground, or stand ground to the pursuer. Escalated fighting may result if the pursuer catches up to the fleeing male or if neither submits; in these cases, one male will bite the other on the head, tail, or midsection. The male being bit will then lie still in submission, bite the other male, or break the grip and flee. Eventually, one male will be established as dominant and gain access to the female (Fitch 1958, Carpenter 1960, 1962, Brackin 1978, personal obs.). Based on these behavioral observations, it is reasonable to predict that bite force and locomotor performance, including speed and stamina, are important in determining the outcome of these interactions. Biting and locomotion are also important for males during copulatory episodes with females. In order to mate successfully, a male must be

able to pursue and catch a potentially fleeing, reproductive female, bite down on her flank, and maintain his grip for the duration of copulation (Fitch 1958, Carpenter 1960, 1962, Anderson and Vitt 1990, personal obs.). Due to the relative importance of biting and locomotion in establishing dominance and access to mating opportunities, and because there is large variation in reproductive success among males (Anderson and Vitt 1990), it can be concluded that bite force and locomotor performance are acted upon by sexual selection pressures and thus important in determining reproductive success among males of this species.

The objectives of this study were to 1) describe the seasonal patterns of plasma T levels, bite force, and locomotor performance for *A. sexlineata*, 2) determine if T levels, bite force, locomotor performance, and morphological traits (i.e. head and hindlimb size and shape) associated with performance differ between the breeding season and post-breeding season, and 3) determine if variation in T levels and morphology account for variation in performance. Specifically, I hypothesize that 1) performance capacity and relative head and hindlimb size will be greater in the breeding season when T levels are presumably high, and 2) there will be positive relationships between performance capacities and the size of associated morphological traits and between performance capacities and T levels. These hypotheses were tested across the active season of a large sample of *A. sexlineata* males.

CHAPTER 2

METHODS

Subject sampling and housing

Adult male *A. sexlineata* were captured by noose in Ocala National Forest, Florida from May 2007-September 2007 and April 2008-May 2008. Lizards were sampled at least once a month ($n \geq 11$) during this period (collection dates and sample sizes are shown in Table 1). Sampling sites were predominantly sand pine scrub and longleaf pine stands, each with loose, sandy soil. Scrub stands had recently been logged or burned resulting in reduced canopy and ground cover (see Appendix B for descriptions of primary sampling sites). Population densities at these sites ranged from approximately 1.3 – 10.1 adult males per hectare. All lizards were captured between 09:00 and 16:30 during normal activity period (Fitch 1958, Carpenter 1959). Only adult males (snout-vent length ≥ 54 mm, Fitch 1958, Hoddenbach 1966) were captured and used in this study. From a distance of approximately 3 meters, sex and age-class were determined by the presence of blue ventral coloration (adult males). After capture, sex and age-class were verified when needed by the eversion of hemipenes and taking snout-vent length measurements. Blood samples were taken from lizards for hormone analysis on the same day of capture (see below), then the lizards were placed individually into cloth bags, held in a small cooler to minimize extreme fluctuations in temperature, and transported to the lab within three days of capture.

In the lab, lizards were housed individually in plastic cages. Photoperiod was controlled by an Electric Sun Tracker (Paragon Electric Co.) to reflect natural cycles (30° N latitude), and temperature remained constant at 24°C. Lizards were provided with

water and fed crickets (*Acheta domestica*) ad libitum. After all performance and morphology measurements were conducted, lizards were either given a toe clip and released at their capture site to avoid resampling of the same individuals or euthanized and deposited at the GSU Herpetology Collection. Lizards were collected, handled, and housed in accordance with Florida Fish and Wildlife Conservation Commission Special Purpose Permit WX07348, U.S. Forest Service Special Use Permit (Use Code 422), and IACUC protocol I06035.

Blood sampling

Blood samples were collected during the normal activity period (between 09:00 and 16:30) within four hours of capture (mean = 52.7 minutes). Blood samples of approximately 10-100 μ l of whole blood were collected from the postorbital sinus using a heparinized microhematocrit capillary tube (MacLean *et al.* 1974, Lindzey and Crews 1986). Blood samples were immediately centrifuged in the field to isolate blood plasma and held on dry ice until return to the lab. Blood samples were stored in the lab at -20°C until assayed for T content. Only blood samples containing a plasma volume of 1 μ l or more were used for hormone analysis.

Bite force

Bite force trials were conducted within three days of capture, after defecation (determined by inspecting cloth bags and cages), and before feeding in captivity. Trials were conducted in the laboratory between 09:30 and 16:00. Lizards were incubated prior to trials for a minimum of 20 minutes at 37-40°C in order to achieve preferred body temperature (Fitch 1958, Witz 2001). Lizards were induced to bite a pre-calibrated bite force meter (methods described in Anderson *et al.* 2008, McBrayer 2004) immediately

after removal from the incubator. Briefly, the jaws of the lizard were lightly tapped which provoked a gape display and an eagerness to bite. The lizards bit down on two alloy plates (1 mm thick, 1.5 mm apart), with the premaxilla approximately 2 mm from the tip of the plates. Electrical tape was placed on the bite plates to minimize stress on teeth and to estimate the location of the bite. A minimum of three trials were conducted for each lizard, where lizards were rested 30-60 seconds between trials. Each trial often consisted of prolonged and repeated biting, noted by flexion and relaxation of jaw muscles and by fluctuations in resistance outputs. The largest value from all trials was considered maximum bite force and used in analysis. Previous studies indicate that this method of measuring maximum bite force in lizards is highly repeatable within and among individuals (Herrel *et al.* 2001, Anderson *et al.* 2008).

Locomotor performance

All locomotor performance trials were conducted within seven days of capture. Lizards were incubated at 37-40°C prior to trials for a minimum of 20 minutes. Trials were conducted in the laboratory between 09:30 and 16:00. For each trial, the lizard was removed from incubation, placed at a designated starting point, and stimulated to run around a circular raceway (circumference = 5 m; Garland 1993, Cullum 1997, 1998). This raceway was constructed of a circular piece of plywood with 41 cm stiff cardboard walls (diameter of inner wall = 139 cm, diameter of outer wall = 179 cm, width of the track = 20 cm) and artificial turf substrate. Five 1 m interval markers were placed on the raceway walls. Lizards were stimulated to run near maximal speeds by tapping the base of the tail with a broom or by hand. Every 30 seconds after the start of the trial, the distance the lizard had traveled (nearest 1 m) was recorded into a voice recorder. The

trial was terminated when the lizard reached exhaustion. When a lizard traveled less than 1 m despite 10 or more stimulus taps, the lizard was placed on its back. If the lizard was able to right itself, the trial continued; if the lizard lost its righting response, the trial was terminated (Garland 1993, 1994, Cullum 1997, 1998).

In consideration of the varying types of behaviors used by male *A. sexlineata* during aggressive and sexual interactions, three different measurements of locomotor performance were recorded from these trials. Burst distance was calculated as the distance (nearest 1 m) the lizard traveled after 180 seconds (Klukowski 1998). This time was chosen because 1) all lizards were able to run for this duration on at least one trial and 2) the speed at which the lizards ran usually decreased considerably after 180 seconds. Max distance was calculated as the distance the lizard had traveled (nearest 1 m) between the start and termination of a trial. This measurement also has been termed “maximal distance run” (Garland 1993) and “maximal exertion” (Cullum 1997, 1998, 2000). Max time was calculated as the time (nearest 1 second) it took the lizard to reach exhaustion. A value for burst distance, max distance, and max time could all be recorded and calculated from a single performance trial. Three locomotor performance trials were conducted for each lizard, where each lizard was subjected to only one trial per day, and the maximum value for each variable was retained for analysis.

Morphology

External measurements of selected morphological traits were obtained while the lizards were in captivity, usually immediately after a locomotor performance trial. Snout-vent length (SVL) was measured with a clear plastic ruler as the distance from the tip of the jaw to the cloaca. Dial calipers were used to measure head length (tip of jaw to back

of skull), head width (quadrate to quadrate), and head height (lowest point of the head to highest point, anterior to tympanum and posterior to orbit). Dial calipers also were used to measure body width at the pectoral girdle, intergirdle length, pelvis width (dorsal surface measurement), and the lengths of the femur (posterior insertion of femur at inguinal pit to medial knee pit), tibia (medial knee pit to calcaneus), metatarsus (calcaneus to base of fourth toe phalange), and fourth toe (largest phalange, excluding claw). Hindlimb length was calculated as the sum of femur, tibia, metatarsal, and fourth toe lengths.

Hormone analysis

Plasma circulating testosterone levels were measured using an enzyme immunoassay (Rodriguez et al. 2000, Olsson et al. 2007, Gil et al. 2008) with Testosterone EIA kits (Cayman Chemicals, Cat. No. 582701). In this competitive enzyme immunoassay, plasma samples collected from lizards containing unknown concentrations of testosterone, a testosterone-acetylcholinesterase conjugate (tracer), and testosterone antiserum are added to wells containing a mouse monoclonal antibody. Testosterone from plasma samples and the tracer compete for a limited number of testosterone antiserum binding sites. Because the concentration of testosterone from plasma samples varies, while the concentration of tracer is held constant in each well, the amount of tracer able to bind to the antiserum is inversely proportional to the concentration of testosterone in the well. After plasma samples, tracer, and antiserum are added to each well, the resulting complexes bind to the mouse monoclonal antibody, the plate is washed to remove unbound reagents, and Ellman's Reagent is added to each well. The enzymatic reaction between Ellman's Reagent and the bound tracer results in a

product that absorbs strongly at 405 nm. The absorbance values at this wavelength were determined for each well with a microplate autoreader (Bio-Tek Instruments, EL311s). Because the amount of bound tracer determines the concentration of enzymatic product and the absorbance value, and because the tracer and testosterone from samples compete for a limited number of binding sites, the testosterone concentrations from plasma samples are inversely proportional to the absorbance values. A standard curve was created for each assay using standards with known testosterone concentrations and plotting the resulting absorbance values. The equations from these standard curves were used to calculate testosterone concentrations from the absorbance values of the plasma samples.

All standards and samples were run in duplicate, and average calculated concentrations were used in statistical analyses. Due to the small volumes of plasma (1-30 μ l), and because results in a preliminary assay revealed no difference between extracted and unextracted plasma samples (paired t-test: $t_{16} = 1.27$, $p = 0.24$), unextracted plasma samples were used in the assays. Varying dilution ratios (volume of plasma: volume of plasma + EIA buffer) were used for different samples, depending on the volume of available plasma and the season from which the sample was collected, so that the percentage of bound analyte fell within the most sensitive portion of the standard curves (all samples used in analysis fell between 19-81.1%; see Olsson et al. 2007). Plasma volumes ranged between 1-30 μ l and EIA buffer volumes ranged between 120-500 μ l. Testosterone concentrations calculated from standard curves were therefore corrected by multiplying the calculated concentration by the dilution factor. For analysis, a total of four separate assays were used, each containing standards to create a standard

curve, 30 μ l samples from a pool of *A. sexlineata* blood to determine interassay variation, and plasma samples collected from each month. Intra-assay coefficient of variation was 16.91%; inter-assay coefficient of variation was 19.94%.

Statistical analyses

Variables that were not normally distributed (max time and plasma T levels, as determined by Kolmogorov-Smirnov test) were log₁₀ transformed to meet assumptions for parametric tests. To determine the repeatability of locomotor performance trials, Pearson's Product Moment Correlations were used to examine the relationship between the maximum values (used in subsequent analyses) to the second highest values of each locomotor variable for all lizards. Pearson's Product Moment Correlations were used to determine if there was a relationship between circulating T levels and time of day when sampled or amount of time between capture and blood sampling. Pearson's Product Moment Correlations were also used to determine the relationship between the three locomotor performance variables (burst distance, max distance, and max time).

Simple linear regressions were performed for morphometric variables, performance variables, and plasma T levels against SVL to determine the influence of body size on these variables. For the variables where SVL explained a significant amount of variation, residual (size-adjusted) values were calculated from these regressions and used in subsequent analyses.

To describe seasonal patterns of plasma T levels and performance, lizards were grouped into 11 separate cohorts based on the Julian calendar date of when they were collected (Table 1). Mean T levels and performance capacities were calculated for each

cohort, and scatterplots were constructed with these means on the Y-axis and Julian dates on the X-axis.

One-way ANOVAs were used to compare performance variables, morphometric variables, and plasma T levels between seasons. A Kruskal-Wallis test was used to compare variables between seasons when data had unequal variances (determined by Levene test). For ANOVAs comparing seasons, lizards collected from April-July were considered breeding season individuals, and those from August-September were considered post-breeding individuals. This distinction was made because gravid females were observed from April-July. Additionally, previous studies have characterized the *A. sexlineata* breeding season as late spring to early summer (in Oklahoma; Carpenter 1960); from April, when mature sperm is present in male testes, to mid-July, when testicular regression begins (in central Alabama/Georgia; Etheridge *et al.* 1986); and from May to early July, as sexual and aggressive behaviors were observed in these months but not in August or later months (in Oklahoma; Brackin 1979).

Standard Least Squares Multiple Regressions were used to determine the relationship between morphometric variables and performance capacities. In constructing multiple regression models, performance variables (bite force, burst distance, max distance, max time) were used as the dependent variable, with size-adjusted morphometrics (head width, head length, and head height for bite force; pelvis, femur, tibia, metatarsus, fourth toe, intergirdle length, and body width for locomotor performance) as independent variables, using only males sampled during the breeding season in analysis.

Pearson's Product Moment correlations were used to examine the relationship between T levels and performance across seasons by analyzing data of lizards sampled from both seasons grouped together. Pearson's Product Moment correlations were also used to examine the relationship between T levels and performance within the breeding season by only analyzing data of lizards sampled from the breeding season. Excluding ANOVAs involving multiple comparisons between months (where $\alpha = 0.003$), p-values < 0.05 were considered significant. All statistical tests were carried out using JMP 7 (SAS Institute).

CHAPTER 3

RESULTS

The maximum and second highest values of all locomotor performance variables were strongly correlated, indicating high repeatability of these measurements (burst distance: $r = 0.87$, $df = 124$, $p < 0.0001$; max distance: $r = 0.91$, $df = 129$, $p < 0.0001$; max time: $r = 0.85$, $df = 129$, $p < 0.0001$). Locomotor performance variables were significantly and positively correlated with each other (burst distance with max time: $r = 0.49$, $df = 131$, $p < 0.0001$; max distance with max time: $r = 0.80$, $df = 131$, $p < 0.0001$; max distance with burst distance: $r = 0.88$, $df = 131$, $p < 0.0001$; see Fig 1).

Plasma T levels ranged from less than 1 ng/ml in several lizards (particularly those sampled in the post-breeding season) to over 21 ng/ml in a lizard sampled in late April. Circulating plasma T levels were not related to the time of day when lizards were bled ($r = -0.06$, $df = 80$, $p = 0.6132$). Circulating T levels were not correlated to the amount of time between when lizards were captured and bled ($r = -0.04$, $df = 80$, $p = 0.7262$).

Snout-vent length (SVL) explained a significant amount of variation (with positive relationships) in all performance and morphometric variables ($p \leq 0.0007$; Table 3). Plasma T levels however were not related to SVL ($p = 0.7575$). Therefore, residual (size-adjusted) performance and morphometric variables, but not T levels, calculated from regressions on SVL were used in subsequent analyses.

In qualitatively describing the seasonal patterns of bite force, max distance, and max time, performance capacities were relatively low in April at the onset of the breeding season, increased and were at their greatest in June and July at the height of mating

activity, and thereafter decreased in the post-breeding months of August and September (Fig 2A,C,D). Burst distance capacity was also relatively low at the onset of the breeding season and peaked at the height of mating activity; however increased burst distance capacity remained for the duration of the active season into August and September (Fig 2B). Plasma T levels were already elevated at the onset of the breeding season, remained elevated for the remainder of the breeding season, and decreased and were at their lowest in the post-breeding month of September (Fig 3).

In testing for seasonal differences in performance and T levels, size-adjusted bite force was greater in the breeding season than in the post-breeding season ($F_{1,143} = 23.46$, $p < 0.0001$; Fig 4A). Neither size-adjusted burst distance nor size-adjusted max distance differed between seasons ($F_{1,131} = 0.06$, $p = 0.8018$, Fig 4B; $F_{1,131} = 2.04$, $p = 0.1556$, Fig 4C). Size-adjusted max time was greater in the breeding season than in the post-breeding season ($F_{1,131} = 14.02$, $p = 0.0003$; Fig 4D). Plasma T levels were higher in the breeding season than in the post-breeding season ($F_{1,82} = 27.84$, $p < 0.0001$; Fig 5). Size-adjusted head width ($F_{1,143} = 0.02$, $p = 0.8788$), size-adjusted head length ($F_{1,143} = 2.50$, $p = 0.1162$), nor size-adjusted hindlimb length ($H = 1.84$, $df = 1$, $p = 0.1748$) differed between seasons. Size-adjusted head height, however, was greater in the breeding season than in the post-breeding season ($H = 4.24$, $df = 1$, $p = 0.0394$).

A multiple regression analysis on size-adjusted morphology did not produce a significant model for burst distance ($p = 0.5232$), max distance ($p = 0.2292$), or bite force ($p = 0.9076$) (Table 4). A significant model was produced, however, for max time ($p = 0.0005$), with tibia and metatarsal length retained in the model as significant predictors of max time (see Table 4). Tibia length had a negative partial regression coefficient, while

metatarsal length had a positive coefficient, indicating lizards with relatively short tibia and relatively long metatarsals have greater locomotor performance as measured by max time.

In analyzing data throughout the entire active season, plasma T levels were significantly and positively correlated with size-adjusted bite force ($r = 0.23$, $df = 82$, $p = 0.0374$) and max time ($r = 0.27$, $df = 77$, $p = 0.0157$); plasma T levels were not correlated with size-adjusted burst distance ($r = -0.12$, $df = 77$, $p = 0.3520$) or max distance ($r = 0.01$, $df = 77$, $p = 0.9161$) (Table 5). In analyzing only data from within the breeding season, plasma T levels were not correlated with size-adjusted bite force ($r = -0.01$, $df = 57$, $p = 0.9650$), size-adjusted burst distance ($r = -0.08$, $df = 52$, $p = 0.5813$), size-adjusted max distance ($r = 0.05$, $df = 52$, $p = 0.7261$). A trend for a positive relationship, though not significant, existed within the breeding season between plasma T levels and size-adjusted max time ($r = 0.23$, $df = 54$, $p = 0.0887$) (Table 6).

CHAPTER 4

DISCUSSION

The first objective of this study was to describe the seasonal patterns of circulating T levels and performance capacities for behaviors associated with reproduction for males of a non-territorial Scleroglossan lizard, *Aspidoscelis sexlineata*. This study also examined whether or not seasonal differences in T and performance exist and if testosterone levels and morphological traits are related to variation in performance capacities. In addressing these questions, it was found that seasonal variation in bite force and locomotor performance, as measured by max time, exists, and that the seasonal patterns of these traits reflect seasonal changes in circulating T levels. While the exact intermediate mechanism(s) are currently not fully understood, these results support the hypothesis that seasonal variation in performance is related to seasonal changes in T levels.

Measured circulating T levels were not related to the amount of time between capture and blood sampling, indicating that blood samples were obtained before stress from capture altered T levels. In the lizard *Urosaurus ornatus*, T levels of individuals subjected to acute stress for less than four hours did not significantly differ from those of control lizards (Moore *et al.* 1991). Plasma T levels measured in this study (mean = 3.4 ng/ml, range = 0.1 – 21.2 ng/ml) were similar to those measured in two previous studies involving male *A. sexlineata* (less than 1 – over 16 ng/ml; Johnson and Jacob 1984, Grassman and Hess 1992). The small discrepancies between studies are likely a consequence of differences in latitude/climate between populations (Florida in current study vs. Tennessee in others), population attributes (eg. genetics, population densities,

prevalence of disease, predation rates), methods of hormone analysis, or sample sizes. All three studies, however, show a similar seasonal trend for plasma T levels to be elevated during the breeding season months and to decline in post-breeding season months.

Although this is the first study to quantify bite force for *A. sexlineata*, bite forces of similarly-sized lizards are comparable to those measured in this study (*Aspidoscelis* spp., Meyers *et al.* 2002; *Anolis* sp., Herrel *et al.* 2007; *Urosaurus* sp., Irschick and Meyers 2007). The max time values in this study (mean = 264 s, range = 180 – 475 s) are comparable to the results of other studies that quantified stamina in *Aspidoscelis* spp. (range = 162 – 528 s, Cullum 1997; range = 191 – 433 s, Cullum 2000; mean = 218 s, Garland 1993). Likewise, the max distance values from the current study (mean = 76 m, range = 36 – 125 m) are comparable to the results of other studies involving *Aspidoscelis* spp. using similar methods (range = 35 – 69 m, Cullum 1997; range = 49 – 69 m, Cullum 2000; mean = 93 m, range = 58-239 m, Garland 1993).

Overall body size, measured as SVL, accounted for a significant amount of variation in both morphometric and performance variables. These results are not altogether surprising as linear morphological traits typically scale geometrically with body size (Meyers *et al.* 2002, White and Anderson 1994), and organismal function is biomechanically related to the size of associated morphological structures (Arnold 1983, Herrel *et al.* 2001). Previous studies also have found positive relationships for morphological traits, bite force, and locomotor performance with body size (Herrel *et al.* 2001, 2007, Meyers *et al.* 2002, Garland and Losos 1994, Robson and Miles 2000). Numerous studies involving a wide range of taxa (including *A. sexlineata*) have revealed

that social dominance and success in male-male contests are often related to large body size (Carpenter 1960, Marco and Perez-Mellado 1999, Beaugrand *et al.* 1996, Heinze and Oberstadt 1999). This effect of body size on the outcome of agonistic contests is likely due to the ability of body size to influence the performance of behaviors used during these contests. In this study, circulating T levels were not correlated with SVL. Other studies have shown a relationship between T levels and SVL in small, presumably young, male lizards (Hews and Moore 1995, Cox and John-Alder 2005, Husak *et al.* 2007), but no relationship in larger, sexually mature lizards (Amey and Whittier 2000, Husak *et al.* 2007). This difference between age and size classes may be related to differences in the developmental/organizational effects versus the activational effects of T on body size (Hews and Moore 1995). In other vertebrate taxa, steroid hormones, including T, have been demonstrated to regulate body size both in early life stages and in adulthood; however the effect of the hormone on body size may differ depending on life history aspects of the species, including the presence of sexual dimorphism in size (Wade 1976, Crews *et al.* 1985).

In describing the seasonal patterns of plasma circulating T levels, T levels were at their highest in April, just after male lizards emerged from hibernation and at the onset of the breeding season. Plasma T remained elevated for the duration of breeding season months (May-July). Other studies have found peak androgen levels at the onset or even just prior to the onset of mating activity (McKinney and Marion 1985, Borg 1994, Tokarz *et al.* 1998, Amey and Whittier 2000). In post-breeding season months, plasma T levels began to decrease in August and were at their lowest in September. At the collection localities of this study, aggressive and sexual behaviors were most frequent in May, June,

and July (personal obs.); other studies describing the reproductive cycle of male *A. sexlineata* report sexual and aggressive behavior beginning in mid-May and peaking in June, the presence of mature sperm in the lumen of seminiferous tubules occurring only from May-July, and maximal testes mass occurring in June (Brackin 1979, Etheridge *et al.* 1986), indicating reproduction climaxes in May-July even though T levels peak in April. In other lizard species, a decoupling of peak plasma T levels and traits associated with T have been reported. For example, aggressive behavior, mating behavior, presence of mature sperm in seminiferous tubules, and maximal testes mass may not occur until a few weeks or months after peak plasma T levels (Tokarz *et al.* 1998, McKinney and Marion 1985, Arslan *et al.* 1978). Thus, an extended period of elevated T levels may be required before T-mediated traits are expressed, and the timing of elevated T levels is important for the timing of the expression of these traits.

The seasonal trends of bite force, max distance, and max time were similar to each other, yet slightly different than those observed for T. These performance capacities were relatively low after emergence, increased gradually during late spring and early summer, peaked in June and July, and decreased in the post-breeding months of August and September. Burst distance followed a similar pattern, but without a noticeable decrease in August and September. Although not a primary objective of this study, male density and activity were estimated from field data using encounter rates per search effort (number of males observed/amount of time spent searching for males) and revealed that male density and activity were highest in June and July (Fig 6). Thus, maximum performance capacities for bite force, max distance, and max time appear to coincide with the height of mating activity, male competition, and female reproductive condition

(Brackin 1979, Etheridge *et al.* 1986). The findings of this study give evidence that bite force and locomotor performance are important for male-male competition, mating behavior, and, consequently, likely related to reproductive success in male *A. sexlineata*.

The fact that T levels were highest at the onset of the breeding season in April, while performance did not peak until June and July, may be because increased performance capacities (and other T mediated traits that do not coincide directly with peak T levels, see above) require several weeks of elevated T levels and/or multiple intermediate, cascading, physiological events. Additional manipulative studies would be beneficial to determine how long T levels need to be elevated in order to elicit a change in bite force and locomotor performance in male *A. sexlineata* (although previous locomotor endurance studies indicate 18 (Sinervo *et al.* 2000), 14-23 (Klukowski *et al.* 1998), 30 (John-Alder 1994), or more than 9 days (Mills *et al.* 2008), depending on the species and methodology). The timing of when other necessary physiological events occur (eg. upregulation of target tissue receptors, presence of 5 α -reductase for dihydrotestosterone conversion) also may result in the decoupling of peak T and performance; testing these hypotheses would result in a more complete understanding of the physiological pathway by which T potentially mediates performance.

In testing specific hypotheses regarding seasonal differences, mean plasma T levels were significantly higher in the breeding season than in the post-breeding season. These data agree with the patterns observed in males of many other seasonally-breeding temperate species, where elevated levels of plasma T and other androgens correspond with spermatogenesis, increased aggression, development of secondary sexual characters, and sexual behavior associated with breeding (McKinney and Marion 1985, Amey and

Whittier 2000, Wingfield *et al.* 1990, but see Crews 1984). Elevated T levels have been demonstrated to both increase fitness (eg. Increased access to mates and other resources, production of viable sperm) and incur fitness costs (eg. Reduced immune function, increased mortality) (Oppliger *et al.* 2004; see review in Hau 2007). Thus, seasonal differences in T levels are likely related to these fitness trade-offs; elevated T levels should be most advantageous during the breeding season when female mates are receptive and competition between males is high, while the costs associated with elevated T levels outweigh the benefits during non-reproductive periods.

Similar to plasma T levels, bite force and locomotor performance, as measured by max time, were both significantly higher in the breeding season than in the post-breeding season. Other studies also have found increased performance capacities in the breeding season (Garland and Else 1987, John-Alder *et al.* 1997, Irschick and Meyers 2007). These results demonstrate that performance capacities of certain behaviors are plastic in response to seasonal changes in environmental and/or physiological cues. Also, it can be concluded that because biting and locomotion are important for gaining mating opportunities for male lizards, high capacities of these behaviors are presumably related to reproductive success during the breeding season. The current study is important as it indicates that the ability to perform key behaviors used in intrasexual contests is important even in non-territorial species. In contrast to predictions that male-male competition and sexual selection are minimal in species that do not actively defend a home range (Stamps 1983), the results of this and other studies demonstrate that the ability to perform in intrasexual contests and the outcomes of these contests are, in fact, important selection pressures for non-territorial species (Anderson and Vitt 1990, Marco

and Perez-Mellado 1999). Because there was no seasonal variation in burst or max distance it is possible that these traits are not directly related to reproductive success in this species, or that benefits associated with high capacities of these traits exist in both seasons (eg. avoiding predation, capturing prey).

Of the morphological traits measured in this study, only relative head height showed variation across seasons. Because T is known to increase muscle mass (Griggs *et al.* 1989, Bhasin *et al.* 1997), and because relatively large heads are able to contain more jaw musculature (Herrel *et al.* 2001, 2007), the seasonal difference in relative head height is possibly linked to seasonal differences in T and its effects on jaw muscle mass. Additionally, because increased head size (and increased jaw musculature contained within) is related to increased bite force (Herrel *et al.* 2001, 2007, Lappin 2006), change in muscle mass is a likely mechanism that links T to seasonal changes in bite force. No differences between seasons were observed for limb or other head morphometrics measured, hence it is possible that these traits are not related to T or performance. However, it should be noted that the methods used to measure these morphological traits likely described primarily skeletal, rather than muscular, elements. More detailed studies involving muscle dissections to analyze muscle mass and muscle properties are required to further investigate morphology as a potential mechanism linking seasonal changes in T and performance. Although several studies have found changes in muscle properties and endurance resulting from anabolic steroid treatment in mammals (Rogozkin 1979, Haupt and Rovere 1984, Bhasin *et al.* 1997, Van Zyl *et al.* 1995), additional studies that investigate physiological levels of naturally occurring androgens in a broad range of taxa

are needed to determine the degree of evolutionary conservation and fitness implications of the influence of androgens on performance.

One goal of this study was to determine how morphological traits, in addition to plasma T, are related to natural variation in performance. As previously stated overall body size accounted for a significant amount of variation in all performance variables. Raw head morphometrics are related to bite force, and raw hindlimb morphometrics are related to locomotor performance, as demonstrated by both the current (regression results of performance on raw morphometrics similar to those of performance on SVL) and previous studies (Herrel *et al.* 2001, 2007, Bonine and Garland 1999, Garland and Losos 1994). Size-free morphological traits, however, did not explain variation in bite force, burst distance, or max distance. Therefore, these performance variables are influenced more by head and hindlimb size, rather than shape. Size-free morphological traits did explain significant variation for max time, with residual metatarsal and tibia length as the most important variables. Results of previous studies examining the relationship of size-free morphological traits with bite force and locomotor performance have been equivocal, and may vary due to the species and methodologies used (Irschick *et al.* 2006, Lappin *et al.* 2006, Herrel *et al.* 2001, Husak *et al.* 2006a, Miles 1994). The correlation of increased max time with a relatively short tibia and relatively long metatarsus deserves further attention, but may be explained by the energetic costs incurred with varying aspects of limb shape (Reilly *et al.* 2007, McBrayer in review). Regardless, it can be concluded that body size is an important factor in explaining variation in performance because large body size results in increased size of morphological structures biomechanically related to performance. Future studies should investigate the

ontogenetic role of T and its effect on growth to further explore the relationships between T, body size, and performance in *A. sexlineata*.

Plasma T levels were significantly and positively correlated to bite force and max time across both the breeding and post-breeding seasons. These results further support the hypothesis that seasonal differences in bite force and max time are driven by seasonal differences in plasma T. While this is the first study to examine seasonal differences in locomotor performance as they relate to seasonal differences in naturally occurring T levels, previous experiments have shown that male lizards with experimentally elevated T levels have improved locomotor performance compared to controls (John-Alder 1994, 1997, Klukowski *et al.* 1998, Sinervo *et al.* 2000). These results suggest that variation in endurance is associated with variation in T for both, territorial and non-territorial lizards. Several hypotheses exist to explain the mechanisms and physiological pathway as to how T levels are related to seasonal variation in stamina. Increased T levels may affect properties of limb muscles, such as mass, fiber types and size, and enzyme activity (Klukowski *et al.* 1998, Brantley *et al.* 1993, Saborido *et al.* 1991, Garland and Else 1987); increase heart muscle mass or hemoglobin concentration and consequently increase oxygen delivery to muscles involved with locomotion (Thorarensen *et al.* 1996, John-Alder 1994, Garland and Else 1987); act synergistically with other hormones (John-Alder *et al.* 1997, Sinervo *et al.* 2000, Holloway and Leatherland 1998); or influence limb shape by altering the size of skeletal elements (Cox and John-Alder 2005, Holloway and Leatherland 1998). No correlation was found for T levels with burst distance or max distance, suggesting performance capacities of these behaviors are not influenced by T. Previous experimental (Klukowski *et al.* 1998) and correlative (Husak *et al.* 2006a)

studies have found mixed results regarding the ability of T to mediate measures of sprint speed; these differences may be attributed to methodologies or reproductive life history aspects of species used in the studies.

In a study examining naturally occurring T levels and bite force, Husak *et al.* (2007) found that there was a positive correlation between these two traits for one morph of *Anolis carolinensis* in their study population, but no correlation for the other morph. Differences in the relationship between T and bite force for different species and morphs may be related to differences in determinants of male reproductive success and fitness cost/benefit trade-offs that exist between species and morphs (Husak *et al.* 2007). No manipulative experiments have explored the mediation of bite force by elevated T levels, and future studies of this type are obviously warranted.

While circulating plasma T levels were related to inter-seasonal differences in performance, they were not related to intra-seasonal variation in performance among males within the breeding season. In the only other comparable study, Husak *et al.* (2006a) found that plasma T levels were not correlated with sprint speed for male collared lizards (*Crotaphytus collaris*) during the breeding season. Husak *et al.* (2006a) hypothesized that above a certain threshold, variation in T levels has no influence on variation in performance (see also Dittami and Reyer 1983). This hypothesis is certainly plausible; in the current study, significant differences in T levels between the breeding and post-breeding seasons were related to marked differences in bite force and max time, but within the breeding season alone, variation in T levels was not as pronounced and did not explain variation in performance. Alternatively, variation among individuals of when emergence from hibernation occurred and when reproductive condition was achieved

may have resulted in a large variance in T levels and performance within a population at a given time period; this large variance may have contributed to the lack of correlation between T and performance within the breeding season. Future studies investigating seasonal changes within individual males rather than a population may elucidate the relationship between circulating T levels and performance within a season, along with providing insight to the timing of when performance increases in response to elevated T.

Another possible hypothesis as to why significant relationships between T and performance were not found in the breeding season is that a time lag exists between when plasma T levels are elevated and when the effects of elevated T levels on performance are realized. In both studies performance was measured approximately the same time (within one week) as blood samples used to measure plasma T were obtained. In considering the seasonal patterns of T and performance (peak T in April, peak performance in June-July) and the amount of time it takes T to influence other T-mediated traits (multiple weeks or months; see Zimmer and Dent 1981, Lyons *et al.* 1986, Bhasin *et al.* 1997, Cox and John-Adler 2005), it is likely that several weeks of elevated T levels and/or a complex pathway of intermediate physiological events are required to increase performance capacities. Consequently, future correlative and manipulative research should consider this possibility when designing studies.

This study revealed that there is marked seasonal variation in circulating T levels, bite force, and locomotor performance in males of a non-territorial species of lizard. These results suggest that increased T levels and increased performance capacities of behaviors used during mating episodes and male-male contests are likely important for reproductive success in the breeding season for non-territorial species. Additionally, this

study provides further evidence that T is able to mediate seasonal variation in performance capacities of certain behaviors, and that elevated T levels and resulting increases in performance are timed in a way that maximizes reproductive benefits while minimizing fitness costs. While a correlative study of this type is an important first step in demonstrating that a trait is mediated by a hormone (Ketterson and Nolan 1999), manipulative studies are now needed to isolate T and its effects on locomotor performance in non-territorial lizard species and bite force in any species. Future studies should investigate physiological pathways that allow T to mediate performance and determine the length of time required for elevated T levels to affect performance. These studies will result in a more complete understanding of how variation in T levels determines variation in performance, and consequently overall fitness, in male vertebrates.

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Table 1
 Summary of collection dates and sample sizes for male *Aspidoscelis sexlineata* used in this study.

Cohort	Collection Dates	n
1	4/11/08 – 4/12/08	14
2	4/26/08 – 4/27/08	6
3	5/6/07; 5/9/07 – 5/11/07; 5/9/08 – 5/12/08	25
4	5/19 /07 – 5/20/07; 5/27/08 – 5/29/08	16
5	6/8/07 – 6/10/07	7
6	6/17/07 – 6/19/07	18
7	6/25/07 – 6/26/07	15
8	7/6/07 – 7/7/07	11
9	8/17/07 – 8/18/07	10
10	8/31/07 – 9/1/07; 9/7/07 – 9/8/07	18
11	9/15/07 – 9/16/07; 9/29/07	5
Total		145

Table 2

Mean performance variables, morphometric variables, and circulating testosterone levels (± 1 S.E.) in male *Aspidoscelis sexlineata* sampled during the breeding and post-breeding seasons. SVL represents snout-vent length.

	Breeding Season		Post-breeding Season	
	Mean \pm SE	n	Mean \pm SE	n
Bite Force (N)	4.33 \pm 0.13	112	3.78 \pm 0.15	33
Burst Distance (m)	64.6 \pm 1.1	100	67.7 \pm 1.7	33
Max Distance (m)	75.6 \pm 1.7	100	75.2 \pm 2.9	33
Max Time (s)	272.7 \pm 6.2	100	241.1 \pm 10.8	33
SVL (mm)	65.4 \pm 0.5	112	67.6 \pm 0.7	33
Mass (g)	6.59 \pm 0.16	100	7.61 \pm 0.24	33
Head Width (mm)	8.54 \pm 0.09	111	8.88 \pm 0.12	33
Head Length (mm)	16.17 \pm 0.13	111	16.83 \pm 0.16	33
Head Height (mm)	7.26 \pm 0.08	110	7.44 \pm 0.09	33
Pelvis (mm)	6.26 \pm 0.08	93	6.58 \pm 0.09	33
Femur (mm)	12.06 \pm 0.14	100	12.14 \pm 0.15	33
Tibia (mm)	12.76 \pm 0.16	100	13.03 \pm 0.17	33
Metatarsus (mm)	9.14 \pm 0.09	93	9.46 \pm 0.08	33
Fourth Toe (mm)	14.68 \pm 0.12	93	14.98 \pm 0.17	33
Intergirdle Length (mm)	34.87 \pm 0.39	92	36.79 \pm 0.50	33
Body Width (mm)	9.34 \pm 0.09	93	9.85 \pm 0.13	33
Testosterone (ng/ml)	4.12 \pm 0.52	59	1.58 \pm 0.50	25

Table 3

Linear regression equations of performance variables, morphometric variables, and circulating testosterone levels on SVL (snout-vent length) for male *Aspidoscelis sexlineata*. SVL explained a significant amount of variation in all performance and morphometric variables but not in testosterone levels.

Variable	Intercept	Slope	R ²	F (df)	p
Bite Force	-6.52	0.16	0.39	90.65 (1,143)	< 0.01
Burst Distance	-4.74	1.07	0.26	45.41 (1, 131)	< 0.01
Max Distance	-25.44	1.54	0.22	36.24 (1, 131)	< 0.01
log Max Time	2.04	0.01	0.08	11.96 (1, 131)	<0.01
Head Width	-2.29	0.17	0.84	732.93 (1, 142)	< 0.01
Head Length	0.58	0.24	0.86	892.66 (1, 142)	< 0.01
Head Height	-1.71	0.14	0.74	398.84 (1, 141)	< 0.01
Hindlimb Length	7.33	0.63	0.63	210.48 (1,124)	<0.01
Pelvis	-1.89	0.13	0.71	296.53 (1, 124)	< 0.01
Intergirdle					
Length	-5.45	0.62	0.78	436.02 (1, 123)	< 0.01
Body Width	0.45	0.14	0.70	295.42 (1,124)	< 0.01
log Testosterone	0.01	0.00	0.00	0.10 (1, 82)	0.76

Table 4

Results of multiple regression analyses of performance using size-adjusted morphometric variables. Data collected from male *Aspidoscelis sexlineata* sampled during the breeding season were used in analyses. Standardized partial regression coefficients are given as b' .

Model			
Independent variable	F (df)	b'	p
Burst Distance	0.88 (7, 84)		0.52
Pelvis		-0.22	0.13
Femur		-0.13	0.33
Tibia		-0.09	0.51
Metatarsus		0.03	0.85
Fourth Toe		0.34	0.03
Intergirdle Length		0.03	0.79
Body Width		0.15	0.19
Max Distance	1.37 (7, 84)		0.23
Pelvis		-0.19	0.19
Femur		-0.09	0.48
Tibia		-0.21	0.13
Metatarsus		0.24	0.08
Fourth Toe		0.22	0.13
Intergirdle Length		-0.03	0.79
Body Width		0.19	0.11
log Max Time	4.24 (7, 84)		< 0.01
Pelvis		-0.07	0.60
Femur		-0.06	0.59
Tibia		-0.30	0.02
Metatarsus		0.48	< 0.01
Fourth Toe		-0.02	0.90
Intergirdle Length		-0.13	0.22
Body Width		0.17	0.10
Bite Force	0.18 (3, 106)		0.91
Head Width		-0.04	0.81
Head Length		0.04	0.73
Head Height		0.07	0.67

Table 5

Pearson's Product-Moment Correlations of log-transformed circulating testosterone levels with size-adjusted performance variables for all male *Aspidoscelis sexlineata* collected in the study.

Variable	n	r	p
Bite Force	84	0.23	0.04
Burst Distance	79	-0.12	0.35
Max Distance	79	0.01	0.92
log Max Time	79	0.27	0.02

Table 6
 Pearson's Product-Moment Correlations of log-transformed circulating testosterone levels with size-adjusted performance variables for male *Aspidoscelis sexlineata* collected during the breeding season.

Variable	n	r	p
Bite Force	59	-0.01	0.97
Burst Distance	54	-0.08	0.58
Max Distance	54	0.05	0.73
log Max Time	54	0.23	0.09

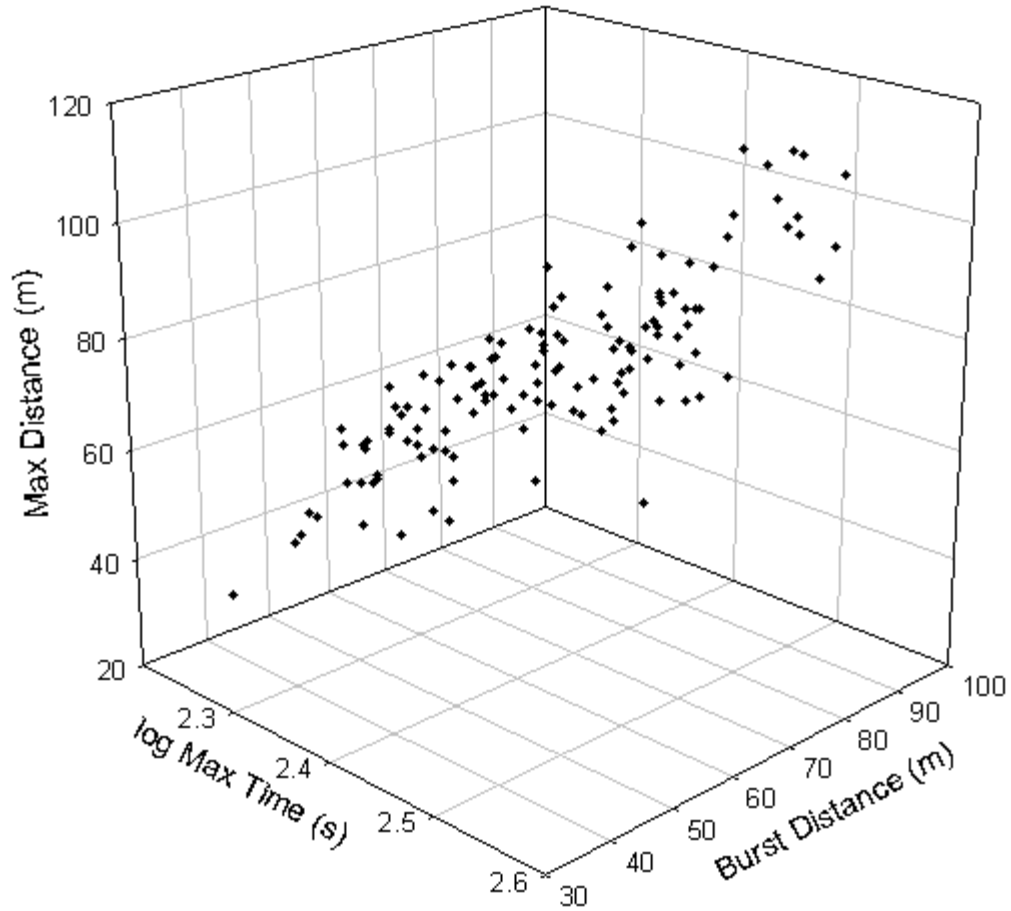


Fig. 1. Three-dimensional scatter plot of three different locomotor performance measurements for male *Aspidoscelis sexlineata*. All variables are significantly and positively correlated ($p < 0.0001$).

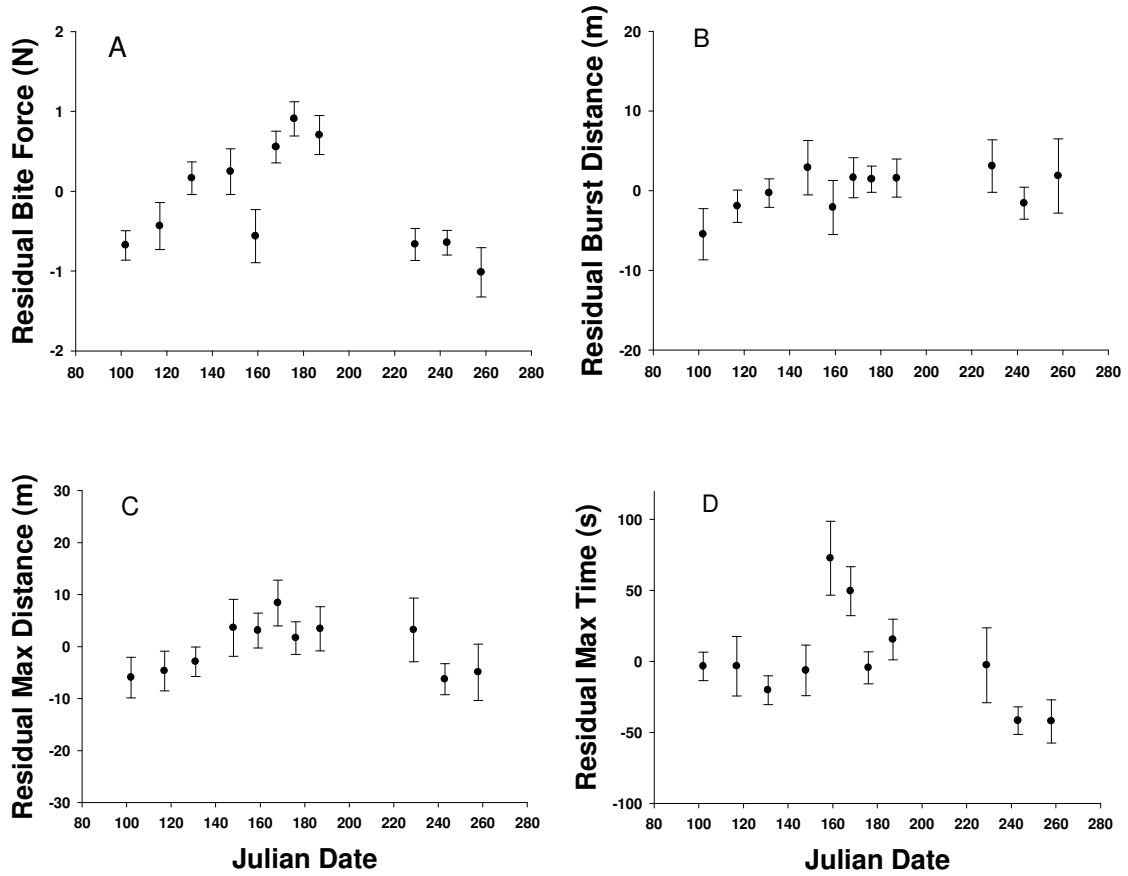


Fig. 2. Seasonal profiles of size-adjusted bite force (A), burst distance (B), max distance (C), and max time (D) performance capacities by Julian calendar date in male *Aspidozelis sexlineata*. Size-adjusted variables were calculated as the residuals of linear regressions for performance variables on SVL (snout-vent length). Data are presented as mean ± 1 SE for each collection cohort.

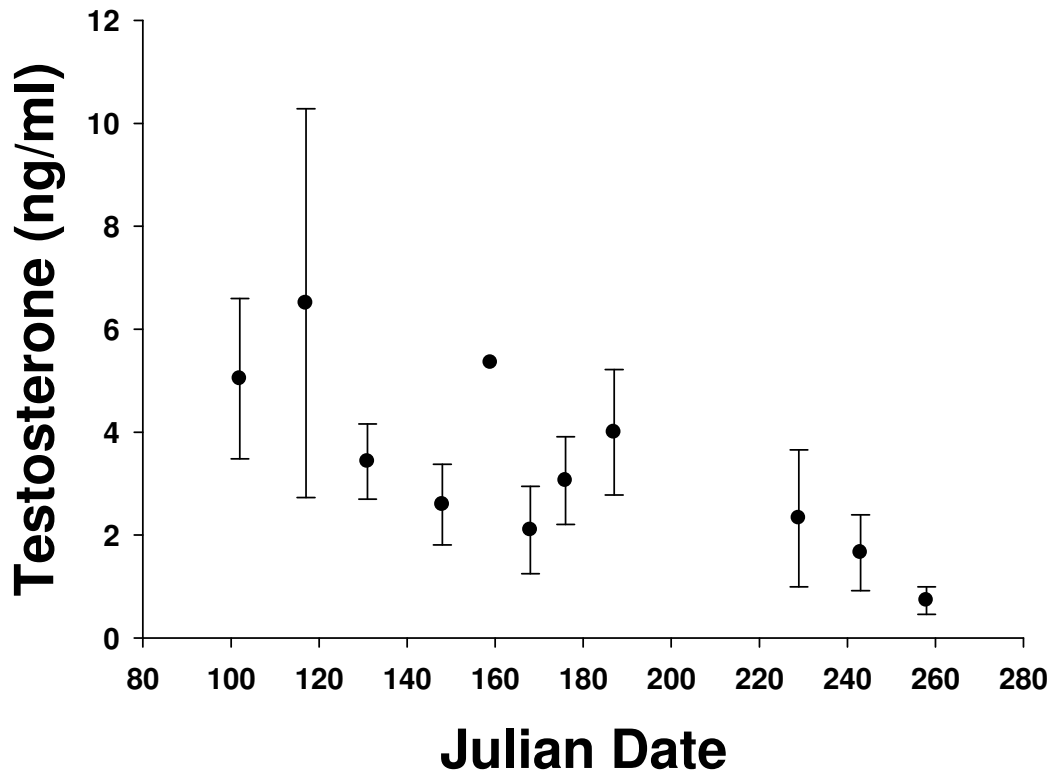


Fig. 3. Seasonal profile of plasma circulating testosterone levels by Julian calendar date in male *Aspidoscelis sexlineata*. Data are presented as mean \pm 1 SE for each collection cohort.

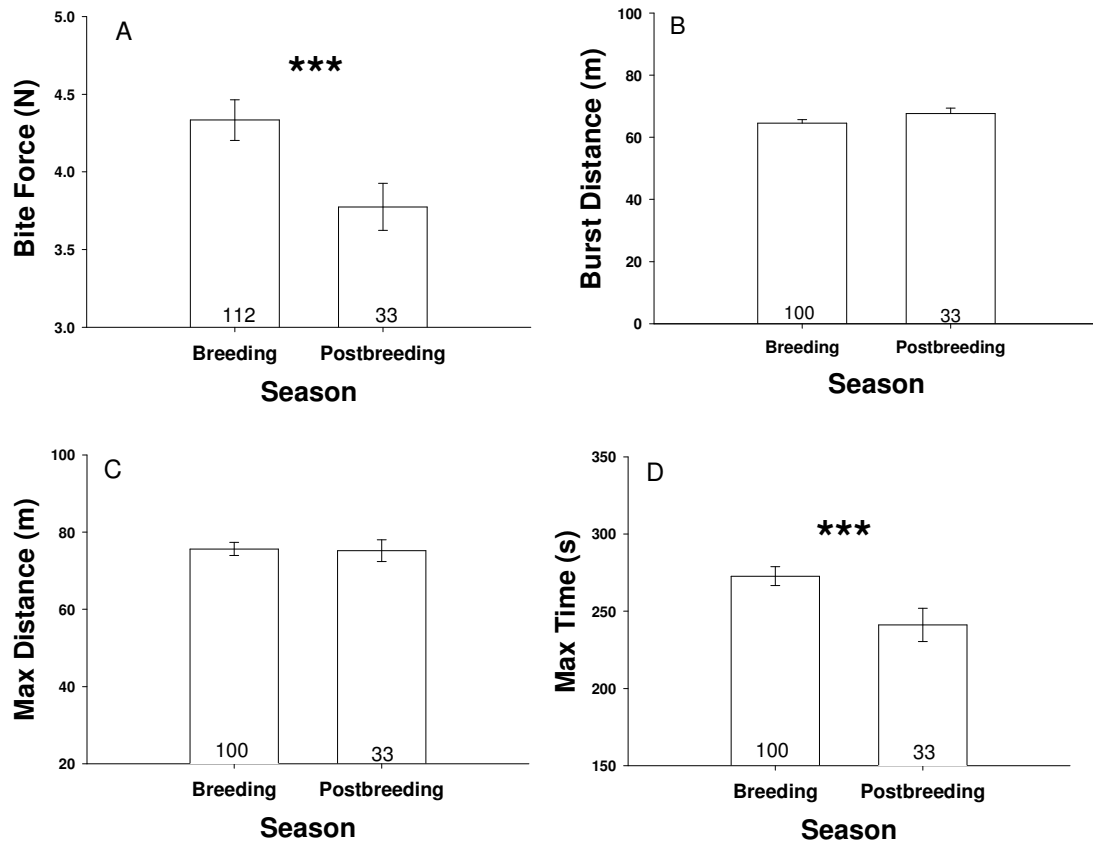


Fig. 4. Seasonal comparisons of bite force (A), burst distance (B), max distance (C), and max time (D) performance capacities in male *Aspidozelis sexlineata*. Data are presented as mean \pm 1 SE. Samples sizes are given at bottom of each bar. *** indicates $p < 0.0001$.

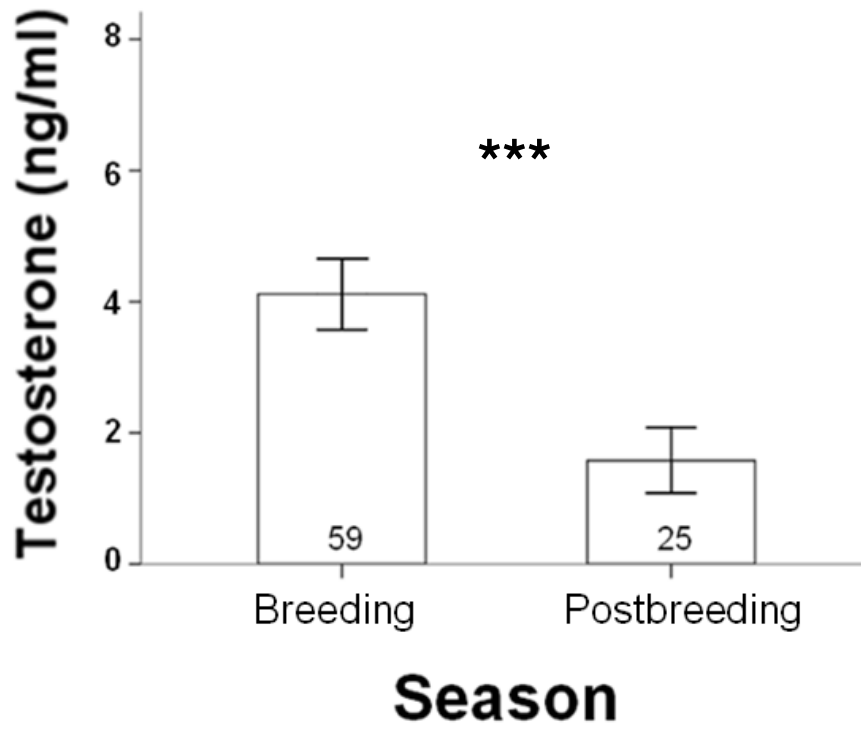


Fig. 5. Seasonal comparisons of plasma circulating testosterone levels in male *Aspidoscelis sexlineata*. Data are presented as mean \pm 1 SE. Sample sizes are given at bottom of each bar. *** indicates $p < 0.0001$.

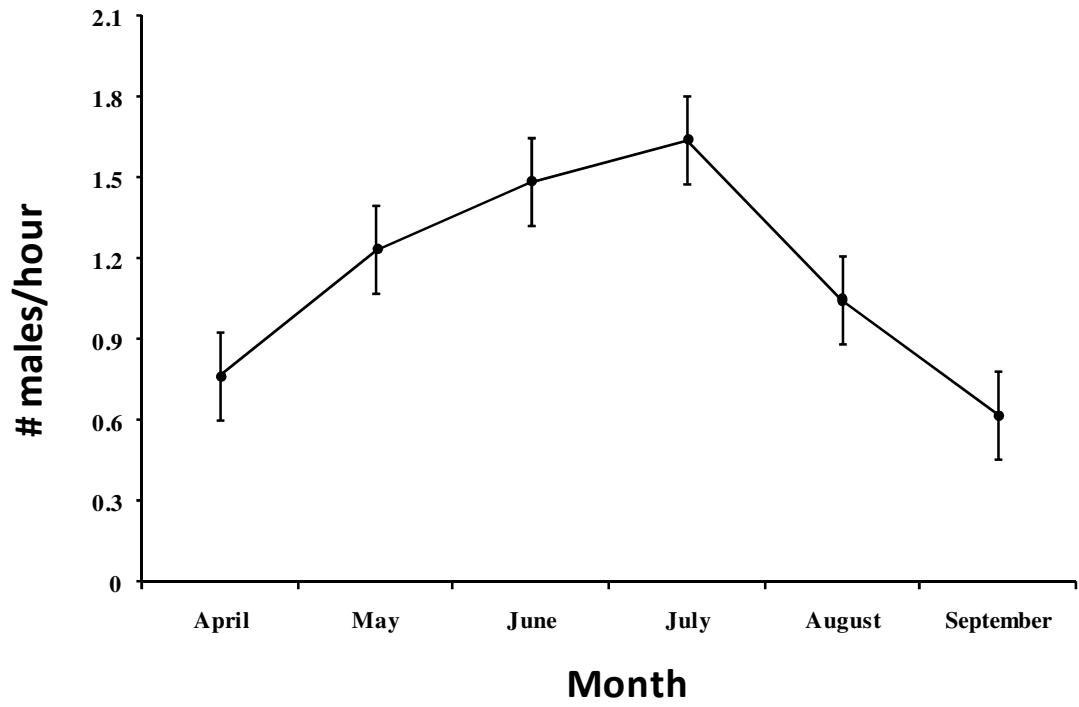


Fig. 6. Mean (± 1 SE) number of male *Aspidoscelis sexlineata* encountered in the field per hour of searching, plotted by month.

APPENDIX A

BROADER IMPLICATIONS OF RESULTS

Seasonal variation in performance

As there were seasonal differences in bite force and max time, it is likely that both costs and benefits exist for maintaining increased capacities of these performance traits and that the relative tradeoffs between these costs and benefits differ between seasons. Increased bite force and locomotor performance are likely beneficial in increasing mating opportunities, and therefore reproductive success, during the breeding season. In context with field observations of male *A. sexlineata* reproductive behavior, locomotor performance and stamina might influence reproductive success by determining the ability to search for mates, endure prolonged agonistic contests, and defend receptive females from competing males, while bite force might determine the outcome of agonistic contests and the ability of a male to successfully copulate with a female (Carpenter 1960, 1962, Leuck 1985, personal obs.). Thus, selection pressures would favor males with increased bite force and locomotor performance in the breeding season.

Because performance capacities decreased in the post-breeding season, however, the mechanism(s) required to maintain increased performance may be costly. While the benefits of increased performance outweigh these costs during the breeding season, the benefits of increased performance are reduced in the post-breeding season when females are not receptive and male competition for mates is less important; thus, the fitness costs associated with maintaining high capacities result in decreased performance during the post-breeding season. If T is indeed a mechanism that mediates performance, it is likely that the costs associated with elevated T levels (increased

mortality, reduced time and energy available for growth and maintenance, and reduced immune function; see review in Hau 2007) are related to decreased performance capacities in the post-breeding season.

Decoupling of elevated testosterone and increased performance

The seasonal patterns of T levels and performance in this study reveal that T is already elevated at the onset of the breeding season in April and remains elevated for the remainder of the breeding season, while bite force and max time reach their peak capacities during the months of June and July. These findings indicate several weeks of elevated T levels may be required to have a significant effect on performance capacities and that this effect likely operates through multiple, cascading events. For example, T may need to be converted to another metabolite (dihydrotestosterone or 17β -estradiol) to be biologically active, bind with target cell receptors to form hormone-receptor complexes, initiate transcription at specific genes such as those regulating muscle production, and promote muscle mass growth for several weeks before a significant change in performance occurs (see Ketterson and Nolan 1999, Hau 2007 for reviews). Additionally, when the seasonal patterns of performance are taken in context with the timing of other events important for male *A. sexlineata* reproduction, such as indices of male-male competition and female receptivity, it appears performance capacities peak when they are most likely to positively influence reproductive success. Therefore, T levels may be elevated at a time (i.e. at the onset of the breeding season) to ensure performance capacities are maximized when they would be most beneficial to reproductive success.

Many manipulative experiments that have used T treatment or implants to examine T-mediated traits did not observe, or even measure, a response in the trait until multiple weeks or months after treatment (Zimmer and Dent 1981, Lyons *et al.* 1986, Bhasin *et al.* 1997, Sinervo *et al.* 2000, Cox and John-Adler 2005). As the performance traits measured in this study are most likely influenced by multiple underlying physiological and morphological factors, it would not be unexpected if changes in these intermediate underlying factors, and consequently performance capacities, required an extended period of elevated T levels. Studies that experimentally manipulate T levels or that further investigate intermediate factors that link T to performance would provide more insight as to why a decoupling of elevated T and increased performance exists.

**APPENDIX B
ATTRIBUTES OF NOTEWORTHY SAMPLING SITES**

	Site		
	Kerr Island	597	Blue Sink
Latitude	29° 21' N	29° 08' N	29° 03' N
Longitude	81° 49' W	81° 46' W	81° 40' W
Estimated Size (ha)	10.4	33.1	11.4
Habitat Type	Longleaf Pine	Scrub Pine	Scrub Pine
Notes	Regularly Burned	Logged, < 5 years old	Logged, < 3 years old
Males Encountered	105	67	15
Search Effort (mins)	4,223	2659	616