
Testosterone Inhibits Growth in Juvenile Male Eastern Fence Lizards (*Sceloporus undulatus*): Implications for Energy Allocation and Sexual Size Dimorphism

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Accepted 11/18/2004; Electronically Published 5/13/2005

ABSTRACT

In the eastern fence lizard, *Sceloporus undulatus*, female-larger sexual size dimorphism develops because yearling females grow faster than males before first reproduction. This sexual growth divergence coincides with maturational increases in male aggression, movement, and ventral coloration, all of which are influenced by the sex steroid testosterone (T). These observations suggest that male growth may be constrained by energetic costs of activity and implicate T as a physiological regulator of this potential trade-off. To test this hypothesis, we used surgical castration and subsequent administration of exogenous T to alter the physiological and behavioral phenotypes of field-active males during the period of sexual growth divergence. As predicted, T inhibited male growth, while castration promoted long-term growth. Males treated with T also exhibited increased daily activity period, movement, and home range area. Food consumption did not differ among male treatments or sexes, suggesting that the inhibitory effects of T on growth are mediated by patterns of energy allocation rather than acquisition. On the basis of estimates derived from published data, we conclude that the energetic cost of increased daily activity period following T manipulation is sufficient to explain most (79%) of the associated reduction in growth. Further, growth may have been constrained by additional energetic costs of increased ectoparasite load following T manipulation. Similar studies of the proximate behavioral, ecological, and physiological mechanisms involved in growth regulation should greatly improve our understanding of sexual size dimorphism.

Introduction

Sexual size dimorphism (SSD) refers to individuals of one sex being characteristically larger than those of the opposite sex for a given population or species. Since the pioneering efforts of Darwin (1871), this widespread biological phenomenon has generally been attributed to differences in the selective forces acting on male versus female body size. However, the leading adaptive hypotheses, which invoke intrasexual selection for large male size and fecundity selection for large female size, fail to explain most of the interspecific variance in SSD among lizards (Cox et al. 2003). Clearly, other factors must be influencing SSD in this group, but what might these factors be?

Adult body size, and thus SSD, is the result of a complex ontogenetic growth process. However, growth is potentially constrained by environmental factors, such as temperature (e.g., Sinervo and Adolph 1989, 1994) and food availability (e.g., Dunham 1978), as well as by energetic costs of daily activity (e.g., Merker and Nagy 1984), territorial behavior (e.g., Marler et al. 1995), and reproduction (e.g., Landwer 1994). Given the numerous adaptive sexual differences in behavior, ecology, physiology, and reproductive investment, growth may often be differentially constrained in males versus females. Thus, SSD may often reflect such constraints rather than adaptive dimorphism in adult body size per se. This conclusion underscores the emerging consensus that the ultimate causation of SSD can be fully understood only with knowledge of the proximate behavioral, ecological, and physiological mechanisms underlying sexual differences in growth regulation and body size (Shine 1990; Watkins 1996; Duvall and Beaupre 1998; Badyaev 2002).

In this study, we manipulated the behavioral and physiological phenotypes of juvenile male eastern fence lizards (*Sceloporus undulatus*) in order to examine the proximate basis of sexually dimorphic growth regulation. In our New Jersey population, males and females hatch in early August at similar sizes (length), and females grow only slightly faster than males until about June of the following summer (age 10 mo). Over the ensuing 3 mo, female growth rate significantly exceeds that of males such that females are 12% larger than males by early September (age 13 mo; Haenel and John-Alder 2002). This

magnitude of female-larger SSD is fully expressed before first reproduction the following spring (age 20 mo) and remains relatively constant among adults thereafter. For clarity, we use the terms “juvenile” and “adult” in reference to animals younger than or older than, respectively, this age of first reproduction. We use the term “maturational” in reference to juveniles during a specific ontogenetic stage (age 10–20 mo) characterized by the gradual development of secondary sexual characteristics and adult behaviors and culminating in reproductive maturity (i.e., age of first reproduction).

The sexual growth divergence that produces SSD under natural conditions is not observed under favorable growth conditions (i.e., ad lib. food, optimal thermal environment) in the laboratory, where juvenile males and females grow at similar rates up to the size of reproductive maturity in the field (Haenel and John-Alder 2002). Further, among juveniles, free-living females grow at a rate comparable to lab-reared females, while free-living males grow considerably more slowly than lab-reared males (Haenel and John-Alder 2002). Thus, it can be inferred that free-living juvenile females are growing at or near their physiological/genetic growth potential, while free-living juvenile males are growing well below theirs. Together, these lines of evidence suggest that environmental factors predominate in the establishment of SSD in *S. undulatus* and raise a question of central importance with regard to the proximate causation of SSD: what factors inhibit juvenile male growth under natural conditions?

Sexual growth divergence among free-living *S. undulatus* is roughly correlated with maturational increases in male aggression, daily movement, and ventral coloration (Skelly and John-Alder 2002; S. Skelly, unpublished data). This observation has two important implications. First, growth and associated functions (e.g., foraging, digestion) require time and/or energy, as do other important organismal functions (e.g., aggression, movement). Therefore, if time and/or energy are limited, growth might be traded off against these competing functions. Second, the sex steroid testosterone (T) influences aggression, daily activity period, movement, and ventral coloration in phrynosomatid lizards (Marler and Moore 1989, 1991; DeNardo and Sinervo 1994; Quinn and Hews 2003; Klukowski et al. 2004). Thus, we hypothesized that female-larger SSD in *S. undulatus* develops because of juvenile male growth inhibition accruing from energetic costs of T-mediated, maturational increases in daily activity period and movement.

We tested this hypothesis by manipulating circulating T levels in field-active juvenile male *S. undulatus* during the period of maximal sexual growth divergence. We predicted that an experimental reduction in circulating T (via surgical castration) would cause a reduction in time and energy allocated to activity and movement, thereby promoting growth. Conversely, we predicted that a compensatory increase in circulating T (via surgical T implant in castrated animals) would increase time and energy allocated to activity and movement, thereby inhibiting

growth. We also included intact control males and females in our experimental design to facilitate the interpretation of our results in the context of sexually dimorphic growth regulation and SSD. To gain insight into specific energetic mechanisms for any observed sex and/or treatment effects on growth, we quantified daily activity period, movement, home range area, spontaneous feeding, ectoparasite load, and body temperature in field-active animals, as well as appetitive food intake in the same animals under controlled lab conditions.

Material and Methods

Experimental Design

We present the results of two experiments, conducted in consecutive summers (2001 and 2002) for replication. Where applicable, we explain minor differences in methodology and experimental design between these two studies.

Animals. Animals were collected under permit from the New Jersey Department of Environmental Protection, Division of Fish and Wildlife (permits SC 21042, 22047, and 24053). All experimental procedures were reviewed and approved by the Rutgers University Animal Care and Facilities Committee (protocol 01-019). We collected lizards from several locations in the vicinity of the Rutgers University Pinelands Research Station in New Lisbon, Burlington County, NJ (41°N, 74°35'W). We captured experimental animals by hand or handheld noose in late May, just before the onset of pronounced sexual growth divergence. We held animals in the laboratory at Rutgers University (see “Laboratory Appetite” for housing conditions) until experimental treatments were completed. In both 2001 and 2002, we assigned males to one of three size-matched treatment groups: (1) castrated, (2) testosterone, and (3) control (see “Surgical Treatments”). In 2002, we included a female group for comparison with control males. We gave each animal a unique toe clip for permanent identification and a dorsal paint mark for undisturbed visual identification during censuses and behavioral observations. We released the animals simultaneously, within several days of treatment.

Testosterone Implants. We constructed tonic-release T implants from 5-mm lengths of Silastic tubing (Dow Corning, 0.058-inch i.d., 0.077-inch o.d.). After sealing one end of each tubule with silicone adhesive gel (NuSil Technology, MED-1037), we used a Hamilton syringe to inject 3 μL of a solution of T (Sigma, T-1500) dissolved in dimethyl sulfoxide (DMSO, 100 μg T μL^{-1}) into the open end of each implant. We then sealed each tubule with silicone adhesive and waited several days for the DMSO to diffuse through the tubing and evaporate, leaving 300 μg of crystalline T within the lumen (ca. 1.5 mm length) of each implant. We constructed placebo implants in identical fashion but injected them with pure DMSO, which left an empty tubule after diffusion and evaporation.

Surgical Treatments. We anesthetized animals with an intramuscular injection of ketamine (Ketaset, Fort Dodge Laboratories, 130 mg kg⁻¹). We then exposed the testes via bilateral or medial ventral incisions and bilaterally castrated (orchietomized) both castrated and testosterone males by ligating each spermatic cord with surgical silk, ablating each testis, and cauterizing each ligated spermatic cord after removal of the testes. For control males and females, we performed “sham” surgeries in which we made identical incisions to expose and manipulate the testes and ovaries while leaving the gonads completely intact. We then inserted either a T implant (testosterone group) or a placebo implant (castrated, control, and female groups) into the coelomic cavity and closed the incision(s) with surgical silk sutures (2001) or Nexaband (Veterinary Products Laboratories) surgical glue (2002).

Field Enclosure. We released the experimental animals into an enclosed area of natural habitat at the Rutgers University Pinelands Research Station (see Niewiarowski and Roosenburg 1993). The enclosed area measured ca. 3000 m² in 2001 but was expanded to ca. 6000 m² in 2002 in order to accommodate a greater number of animals. All resident (nonexperimental) animals were removed from the enclosure before each experiment. Initial and final lizard densities within the enclosure were comparable to natural densities of juveniles measured in the adjacent, free-living population (Niewiarowski and Roosenburg 1993; S. Skelly, unpublished data).

Treatment Verifications

Plasma Testosterone. To examine the effect of our manipulations on circulating T levels, we used heparinized microhematocrit capillary tubes (Fisher Scientific) to collect blood from the post-orbital sinus of each experimental animal at the end of the 2002 study (45 d postrelease). To examine long-term treatment effects, we collected additional samples from those males that survived until July 2003 (418 d postrelease). We also sacrificed and dissected all animals recaptured in 2003 to verify our castration surgeries. To compare our experimental plasma T values with natural levels, we collected blood samples from free-living juvenile males and females in 2004. We sampled the natural population at four ontogenetic stages: ages ca. 2 mo (October), 9 mo (May), 11 mo (July), and 14 mo (October). Owing to the small size of juveniles at 2 mo posthatching (1–2 g body mass), we collected blood samples via decapitation at this age. All blood samples were collected from animals within 2 min of capture. Hereafter, we distinguish between experimental versus free-living animals when discussing plasma T levels.

We held blood samples on ice until they could be centrifuged (within 6 h of collection) and stored the separated plasma at –20°C until subsequent assays. We performed radioimmunoassays for plasma T following the methods of Smith and John-Alder (1999). Samples were extracted twice in diethyl

ether, dried under a stream of ultrafiltered air, and reconstituted in phosphate-buffered saline with gelatin. Reconstituted samples were assayed with T antiserum (1 : 18,000 initial dilution) developed in rabbits by A. L. Johnson (University of Notre Dame, IN). Tritiated hormone (³H-T) was obtained from PerkinElmer Life Sciences. Samples from separate years were analyzed in separate assays (typical interassay and intraassay variation of 6% and 7%, respectively; Smith and John-Alder 1999). Limits of detection were 14, 10, and 5 pg T in 2002, 2003, and 2004, respectively. Individuals whose plasma T levels fell below the limit of detection (one experimental female in 2002, five free-living juveniles in 2004) were assigned a potency value equivalent to the limit of detection and included in subsequent analyses.

Ventral Coloration. Adult male *Sceloporus undulatus* exhibit bright blue throat and abdominal patches, but this blue ventral coloration is absent or greatly reduced in adult females and juveniles of both sexes. Juvenile males develop this coloration over the period in which we performed our experiments (S. Skelly, unpublished data), and T is known to influence the expression of blue ventral coloration in this species (Quinn and Hews 2003). Thus, as an additional corroboration of the physiological response to our experimental manipulations, we recorded blue ventral coloration upon recapture of each animal as a categorical value between 0 (no coloration) and 5 (typical adult male coloration).

Response Variables

Growth Rate. Following release, we recaptured experimental animals at 2-wk intervals and measured snout-vent length (SVL) to the nearest 1 mm and body mass to the nearest 0.1 g. We defined growth as change in length and estimated growth rate for each animal as the slope of the linear regression of SVL on elapsed time (mm d⁻¹). We made recapture measurements over periods of 56 and 45 d postrelease in 2001 and 2002, respectively. Additionally, we recaptured a subset of surviving 2002 experimental males in July 2003 and measured SVL and mass (as well as ventral coloration and plasma T, see “Treatment Verifications”) at this time (418 d postrelease) to examine long-term treatment effects on growth.

Activity Period, Movement, and Home Range. We conducted enclosure censuses on a total of 16 and 12 d in 2001 and 2002, respectively, spanning the entire interval over which we measured growth. On these days, we systematically traversed the entire enclosure every 1 (2001) or 2 h (2002, owing to the larger enclosed area) from 0800 to 2000 hours, which encompassed the entire activity period of our animals. Censuses were not conducted on days when inclement weather curtailed activity for any portion of the day. During each census, we noted the location of each visible (active) animal to the nearest

1 × 1 m on a coordinate grid. We calculated daily activity period (h d^{-1}) for each animal as the total time from earliest to latest sighting of that animal on a given day.

We also used these census data to measure the distance traveled by active animals. We calculated minimum daily movement (m d^{-1}) as the sum of linear distances between consecutive sightings of an animal in a given day. This measure of minimum displacement from one sighting to the next underestimates the actual distance traversed by an animal in that time, so we corroborated this conservative estimate with continuous focal observations of movement (see “Spontaneous Movement and Feeding”). Our measures of daily activity period and minimum daily movement yielded one data point per individual per census day. We did not observe any systematic temporal trends in these measures within individuals, so we combined the 16 (2001) or 12 (2002) daily measurements into a single mean value for each animal.

We used Homerange Analysis and Graphics Package (version 6.07.91, P. Niewiarowski and A. Dunham, unpublished software) to construct minimum convex polygon (MCP) estimates of home range area (m^2) for each animal in our 2002 study. We verified that our MCP estimates were independent of number of sightings ($N > 20$ for all animals) before subsequent analyses. Our experimental animals were transplanted from their natural home ranges into an enclosed area of similar habitat, so our MCP estimates should be interpreted as descriptions of two-dimensional spatial use within this enclosed area rather than home ranges in the classic sense.

Spontaneous Movement and Feeding. In 2001, we conducted continuous, 10-min focal observations of every animal on each of 8 d spanning the experimental period. Observations were made with binoculars from a distance of at least 8 m to reduce potential observer effects and were conducted under both clear and cloudy skies between 1000 and 1600 hours. We recorded the total distance traversed by the animal in each focal period and estimated spontaneous movement (m h^{-1}) by combining all focal observations for a given animal. To produce an estimate directly comparable to our census measure of minimum daily movement, we calculated estimated daily movement (m d^{-1}) as the product of spontaneous movement (m h^{-1}) and daily time abroad (h d^{-1}) for each animal.

We also recorded the number of times that an animal struck and (apparently) consumed a prey item in each focal period. In several instances where an animal successively consumed several small prey items (ants or termites), we scored the multiple strikes as a single meal and estimated spontaneous feeding (meals h^{-1}) by combining all focal observations for a given animal.

Ectoparasite Load. In 2002, we used a hand lens to count visible ectoparasites at each recapture. We observed trombiculid mite larvae (chiggers), lone-star ticks (*Amblyoma americanum*), and

black-legged ticks (*Ixodes scapularis*) on our experimental animals. Tick parasitism was infrequent, so we quantified ectoparasite load as the number of mites visible at each recapture.

Body Temperature. In 2002, we measured the body temperature (T_b) of active lizards on 7 d over the course of our experiment, with each animal measured no more than once daily. We used a fast-reading thermometer (Miller and Weber) to record cloacal temperatures of active animals captured without a chase between 1000 and 1700 hours. We did not observe an effect of time of day on T_b , so we combined all measurements into a single mean value for each animal.

Laboratory Appetite. At the conclusion of the field measurement period in 2002 (45 d postrelease), we returned all experimental animals to Rutgers University, where they were housed individually in 10-gal glass aquariums. To ensure social isolation, we visually separated all cages with opaque barriers. Animals were maintained on a 12L : 12D photoperiod and had access to a 25-W incandescent lamp for basking during photophase. Thermal gradients within each cage ranged from 25° to 45°C during photophase and averaged 19°C during scotophase. Water was available ad lib. We fasted animals for 2 d following introduction to the lab cages and initiated feeding trials on day 3 by stocking each cage with five crickets (*Acheta domestica*) of similar size. We searched each cage for uneaten crickets at 1000 and 1400 hours on each of the four subsequent days and replaced any consumed crickets at this time such that each cage always contained five crickets. We assumed that missing crickets had been consumed and measured daily food intake as an estimate of laboratory appetite (crickets d^{-1}) for each animal.

Statistical Analyses

All statistical analyses were conducted using SAS (version 8.2, SAS Institute). Before statistical analyses, we \log_{10} transformed home range area and ectoparasite load to normalize their distributions. We did not observe significant allometry in any of our variables, so we did not include body size as a covariate in any subsequent analyses testing for treatment and sex effects.

Treatment Effects. When we measured a given response variable in only one year, we tested for treatment effects among male groups with a one-way ANOVA model containing treatment as the main effect. We observed systematic temporal variance in ectoparasite load, so we tested for treatment and time effects with repeated-measures ANOVA. We analyzed our categorical measure of throat coloration with a nonparametric Kruskal-Wallis ANOVA (SAS Institute 1989). When we measured a response variable in both 2001 and 2002, we employed a two-way ANOVA with treatment and year as main effects. When we observed significant treatment effects, we used the Ryan-Einot-Gabriel-Welsch test (REGWQ, SAS Institute 1989) to de-

termine post hoc separation of treatment groups. We could not assume linear growth rate over the 418-d period between initial 2002 release and recapture in 2003, so we tested for a long-term treatment effect on growth by performing an ANCOVA with treatment as the main effect, final SVL in 2003 as the dependent variable, and initial SVL in 2002 as the covariate.

Sex Effects. Using data from 2002, we tested for sex effects by comparing response variables for control males and females in a one-way ANOVA with sex as the main effect. We used repeated-measures ANOVA and nonparametric Kruskal-Wallis ANOVA for the analysis of ectoparasite load and coloration, respectively. On the basis of our previous findings in free-living juveniles, we predicted that control males would exhibit reduced growth and elevated daily activity period, movement, and home range size relative to females (S. Skelly, unpublished data). Therefore, we used one-tailed tests to interpret sex effects on these variables. We did not include females in any of our analyses involving castrated and testosterone males because the absence of complementary female treatments would have precluded valid inferences with regard to the source (i.e., treatment vs. sex) of any observed effects.

Results

Treatment Verifications

Plasma Testosterone. Plasma T was low and did not differ between free-living juvenile males and females at 2 mo post-hatching (Fig. 1A). However, by 11 mo of age (July), plasma

T in free-living males reached a seasonal peak and was significantly higher than in females. Our experiments were conducted during this period of sexual divergence in plasma T, which also coincides with natural sexual divergence growth rate.

In experimental animals, plasma T was significantly higher in testosterone males relative to both castrated and control males (Fig. 1B), but plasma T levels did not differ significantly in control males relative to either castrated males or females. Upon dissection, we did not find any discernible gonadal tissue in either castrated or testosterone males ($N = 7$), which suggests that our surgical castrations were successful. All experimentally induced plasma T levels were well within the natural physiological range of circulating T observed in free-living juvenile males of similar age (Fig. 1). However, while plasma T was as low in some individual free-living males as in experimental control males, mean plasma T was substantially lower in experimental controls than in free-living males.

Ventral Coloration. Overall, we observed a progressive increase in the expression of blue ventral coloration during the course of our experiments. This progression was accelerated by T and retarded by castration such that we found significant treatment effects on ventral coloration at 56 (2001) and 45 d (2002) postrelease (Table 1). We also observed significantly more ventral coloration in control males relative to females at this time (Table 1). At 418 d postrelease, all testosterone and control males exhibited fully developed, adult male ventral coloration, while all castrated males exhibited weak coloration similar to that of adult females (Table 1).

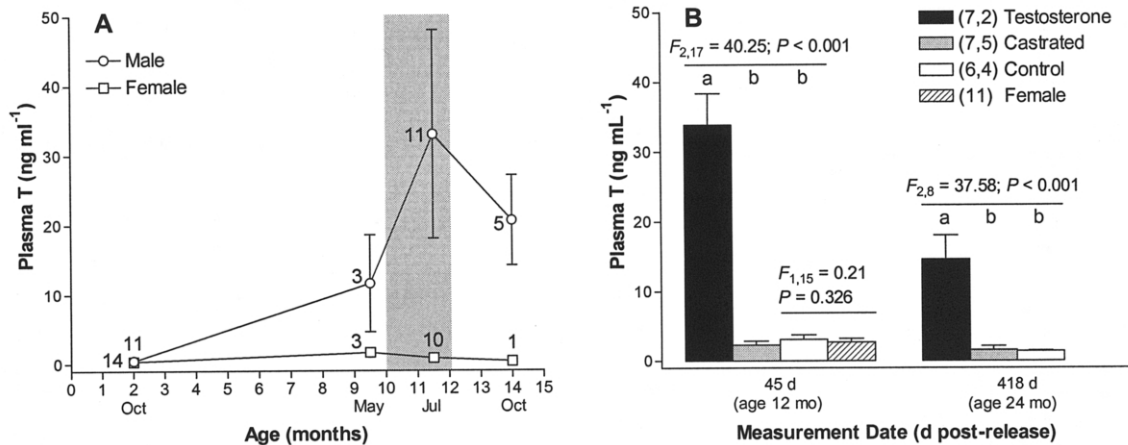


Figure 1. Mean (\pm SE) plasma T levels (A) versus age for free-living juvenile male and female *Sceloporus undulatus* and (B) versus measurement date for experimental treatment and sex groups. A, Numbers above and to the left of symbols indicate sample sizes for females and males, respectively. The shaded area indicates the approximate timing and duration of the 2001 and 2002 experiments. Free-living males and females did not differ in plasma T at age 2 mo ($F_{1,23} = 0.28, P = 0.298$, one-tailed), but males had significantly higher plasma T levels at age 11 mo ($F_{1,19} = 4.67, P = 0.022$, one-tailed). B, Numbers in parentheses indicate sample sizes at 45 and 418 d, respectively. ANOVA revealed a significant effect of male treatment on plasma T at both 45 and 418 d, but control males and females did not differ in plasma T. Lowercase letters denote post hoc statistical separation of male groups (Ryan-Einot-Gabriel-Welsch test). Note that experimentally induced plasma T levels (testosterone group) are comparable to levels observed in free-living males of similar age, but plasma T levels in control males are atypically low.

Table 1: Ventral coloration for *Sceloporus undulatus* sex and treatment groups

Time of Measurement and Treatment Group	Ventral Coloration (Score)	
	N	Mean \pm 1 SE
2001, 56 d postrelease:		
Testosterone	7	2.86 \pm .40
Castrated	8	.88 \pm .23
Control	6	3.33 \pm .42
2002, 45 d postrelease:		
Testosterone	8	3.25 \pm .19
Castrated	9	.44 \pm .13
Control	6	1.67 \pm .54
Female	12	.13 \pm .07
2003, 418 d postrelease:		
Testosterone	2	5.00 \pm .00
Castrated	5	2.20 \pm .58
Control	4	5.00 \pm .00

Note. Nonparametric Kruskal-Wallis ANOVA revealed a significant effect of male treatment on ventral coloration at 56 d in 2001 ($\chi^2 = 12.67$, $df = 2$, $P = 0.002$), at 45 d in 2002 ($\chi^2 = 15.11$, $df = 2$, $P < 0.001$), and at 418 d in 2003 ($\chi^2 = 12.67$, $df = 2$, $P = 0.002$). Control males exhibited more ventral coloration than females at 45 d in 2002 (Kruskal-Wallis ANOVA; $\chi^2 = 8.64$, $df = 1$, $P = 0.002$; one-tailed).

Response Variables

Survival. Although larger sample sizes would be necessary to detect significant treatment effects on survival, both control and testosterone males tended to experience greater mortality than either castrated males or females. At 56 d postrelease in 2001, we recaptured six of 11 control males (55% survival), seven of 14 testosterone males (50%), and eight of 11 castrated males (73%). At 45 d postrelease in 2002, we recaptured six of 14 control males (43%), eight of 14 testosterone males (57%), nine of 13 castrated males (69%), and 12 of 14 females (86%). Our subsequent analyses of response variables are based on these surviving animals.

Growth Rate. In both 2001 and 2002, we observed a significant reduction in growth rate in testosterone males relative to both castrated and control males (Fig. 2). Although castrated males tended to grow faster than control males in 2001, we did not detect any significant differences in growth rate between these groups over the immediate 45- and 56-d experimental periods. However, we did see a significant long-term effect of castration on growth such that for any given size at release in 2002, castrated males were 3–5 mm larger than control males upon recapture 418 d later in 2003 (Fig. 2D). We also observed slightly higher growth rates in females relative to control males in 2002 (Fig. 2C).

Activity Period. We observed a significant increase in the daily activity period of testosterone males, which were active for about 1.5 h d⁻¹ longer, on average, than castrated and control males (Fig. 3A). We did not observe an effect of sex on daily activity period (Fig. 3A).

Movement. We observed a striking increase in minimum daily movement in testosterone males relative to castrated and control males, which moved similar distances per day (Fig. 3B). We found a qualitatively similar pattern among male treatment groups in 2001 when we employed continuous focal measures of spontaneous movement ($F_{2,18} = 4.35$, $P = 0.029$) and estimated daily movement ($F_{2,18} = 5.75$, $P = 0.012$). Averaged across male treatment groups, minimum daily movement underestimated distance traveled by a factor of 3.52 when compared with estimated daily movement. We did not detect any significant sex difference in minimum daily movement (Fig. 3B).

Home Range. We observed a dramatic treatment effect on home range area such that testosterone males used more enclosure space than control males (Fig. 3C). In turn, control males maintained significantly larger home ranges than castrated males. We also saw a nonsignificant trend toward larger home range area in control males relative to that in females (Fig. 3C).

Ectoparasite Load. Across all groups, mite counts were low at 17 d postrelease, peaked at 31 d postrelease, and had declined to intermediate levels by 45 d postrelease (Fig. 4). Overall, we found significantly more mites on testosterone males than on castrated and control males (Fig. 4). Although sexes did not differ in ectoparasite load across all recapture dates, females had significantly more mites than control males by 45 d post-treatment (Fig. 4).

Food Consumption. We did not find an effect of either treatment or sex on either measure of food consumption (Table 2). We observed feeding strikes on a relatively infrequent basis in undisturbed animals (approximately one meal h⁻¹) such that the lack of a treatment effect on male spontaneous feeding might reflect the considerable resultant variance in this measure. However, we did not detect so much as a trend toward reduced food consumption in testosterone males in either the field or the lab. Similarly, we found no difference in laboratory appetite between control males and females (Table 2).

Body Temperature. We did not detect a difference in active T_b among testosterone (33.09° \pm 0.29°C), castrated (32.78° \pm 0.29°C), or control (32.77° \pm 0.43°C) males ($F_{2,20} = 0.32$, $P = 0.728$). Further, we did not find a difference in T_b between females (32.84° \pm 0.21°C) and control males ($F_{1,16} = 0.03$, $P = 0.870$).

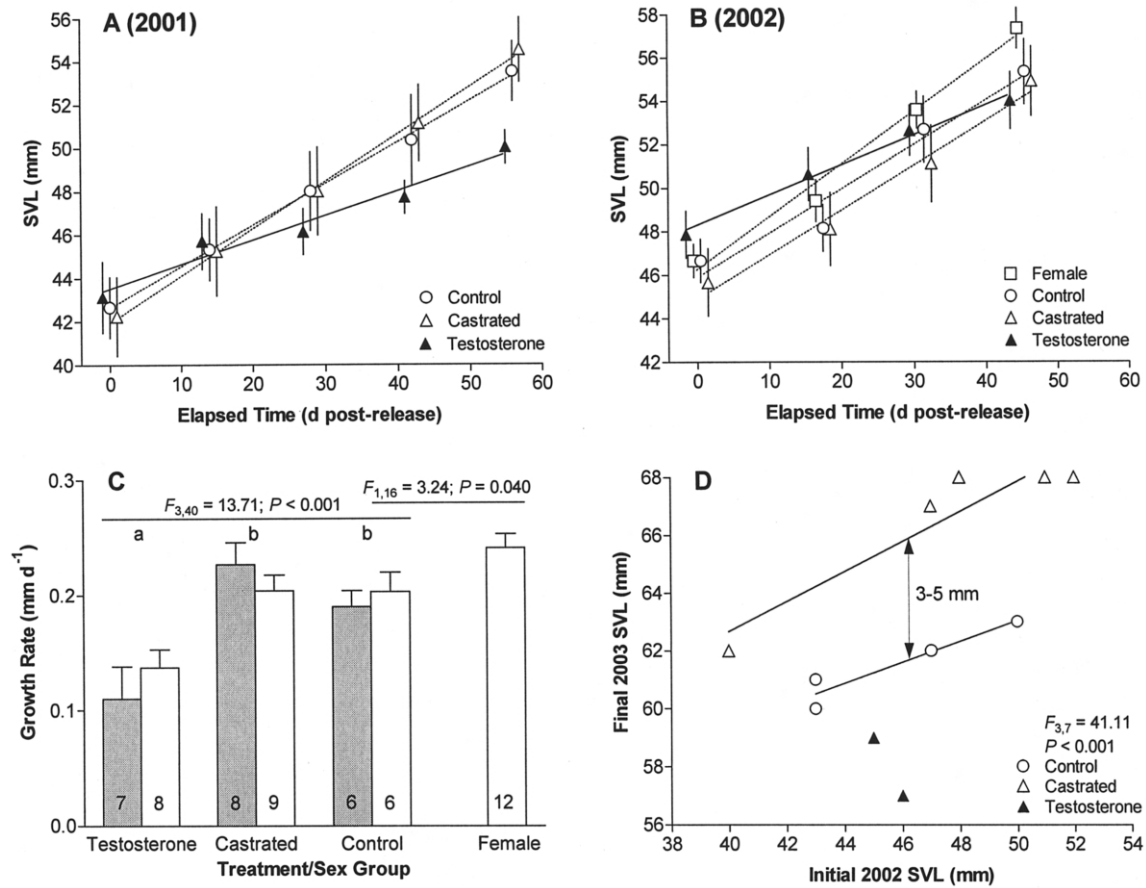


Figure 2. Mean (\pm SE) snout-vent lengths (SVL) versus elapsed time for experimental treatment and sex groups in 2001 (A) and 2002 (B). For visual clarity, symbols are horizontally offset above each time point. Repeated-measures ANOVA of SVL revealed a significant time-by-treatment interaction among males in 2001 ($F_{3,68} = 3.20, P = 0.004$) and 2002 ($F_{6,60} = 6.68, P < 0.001$), and a significant time-by-sex interaction in 2002 ($F_{3,48} = 2.34, P = 0.042$, one-tailed). C, Mean (\pm SE) growth rates (equivalent to the slopes in A and B) for each group in 2001 (shaded bars) and 2002 (open bars). We compared growth rates using two-way ANOVA with year (2001, 2002) and either male treatment (control, castrated, and testosterone males) or sex (control males and females) as main effects. Lowercase letters denote post hoc statistical separation of male groups (Ryan-Einot-Gabriel-Welsch test). Numbers within bars indicate sample sizes. D, Final 2003 SVL upon recapture at 418 d postrelease versus initial 2002 SVL, demonstrating long-term treatment effects on growth. Statistics are reported for ANCOVA with final 2003 SVL as the dependent variable, treatment as the main effect, and initial 2002 SVL as a covariate.

Discussion

Female-larger SSD in *Sceloporus undulatus* develops because yearling females grow faster than males before first reproduction. This sexual growth divergence is subject to strong proximate environmental regulation (Haenel and John-Alder 2002) and occurs roughly coincident with maturational increases in male aggression, movement, and ventral coloration (Skelly and John-Alder 2002; S. Skelly, unpublished data). On the basis of these observations, we hypothesized that female-larger SSD arises because of energetic constraints on juvenile male growth accruing from maturational increases in daily activity period and movement. We further hypothesized that these behavioral

changes in males are at least partially induced by maturational increases in plasma T. Our hormone data from free-living juveniles lend support to this proximate endocrine mechanism; sexual divergence in plasma T roughly coincides with sexual divergence in growth, behavior, and coloration. In turn, our experimental results clearly demonstrate that exogenous T inhibits growth in juvenile males. The physiological relevance of this effect is corroborated by the opposite response (i.e., increased long-term growth) in castrated males. Our results also convincingly show that exogenous T increases daily activity period, daily movement, home range area, and ectoparasite load, each of which may incur an energetic cost that could

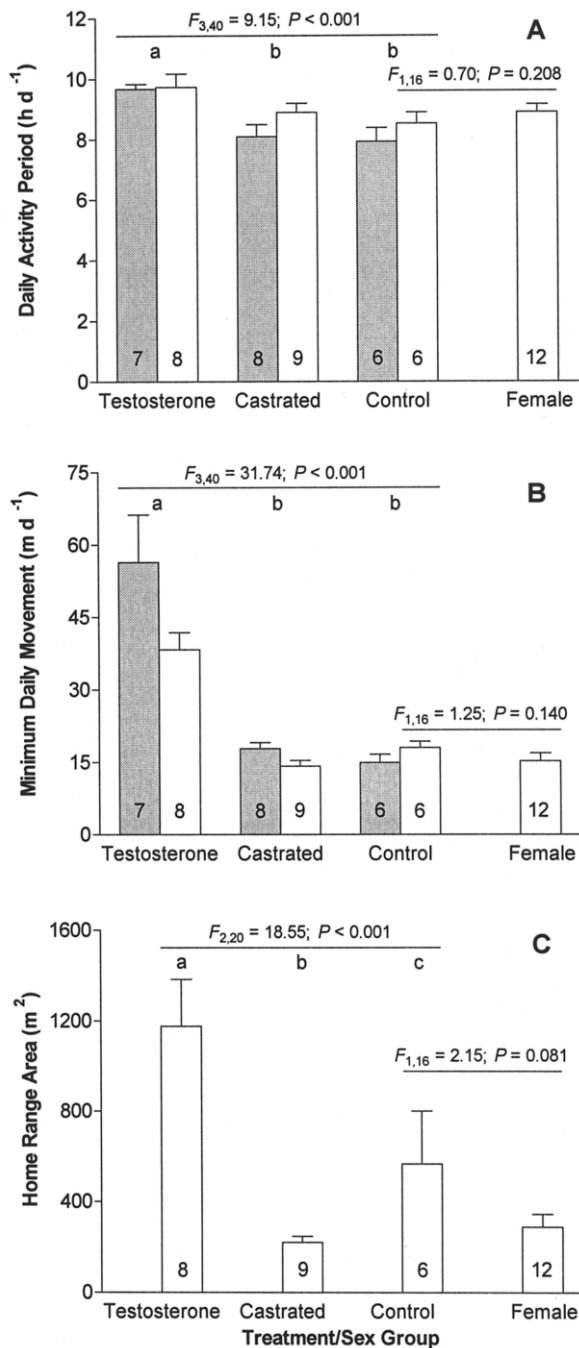


Figure 3. Mean (± 1 SE) daily activity period (A), minimum daily movement (B), and home range area (C) for treatment and sex groups in 2001 (shaded bars) and 2002 (open bars). Numbers within bars indicate sample sizes. For A and B, we made comparisons using two-way ANOVA with year (2001, 2002) and either treatment (testosterone, castrated, and control males) or sex (control males and females) as main effects. For C, we used one-way ANOVA with treatment or sex as the main effect. Lowercase letters denote post hoc statistical separation of male groups (Ryan-Einot-Gabriel-Welsch test). Home range area was \log_{10} transformed before analysis.

potentially constrain growth. In the following paragraphs, we briefly compare our results with those of similar T manipulation studies of growth and then consider their implications with regard to patterns of energy allocation and, ultimately, SSD.

Testosterone and Growth Regulation

Among squamate reptiles, castration and/or T implantation studies have generally shown that T inhibits mass gain (Crews et al. 1985; Marler and Moore 1989, 1991; Klukowski et al. 1998; but see Salvador and Veiga 2000), thereby suggesting an energetic cost of elevated plasma T levels. However, Uller and Olsson (2003) reported an increase in mass gain of neonatal *Lacerta vivipara* following embryonic exposure to exogenous T, which raises the possibility that early organizational effects of T on growth might differ from those observed in manipulations of adult or even juvenile animals. A handful of studies have also demonstrated an inhibitory effect of T on skeletal growth (i.e., change in length rather than mass) in squamate reptiles (Hews et al. 1994; Hews and Moore 1995; Abell 1998; Lerner and Mason 2001; see also Salvador and Veiga 2000). Unfortunately, several of these studies also reported drastically reduced survival among T-implanted animals and acknowledged concerns over possible pharmacological effects of T manipulation. Our experiments did not have this problem because (1) we saw no indication that survival differed between testosterone and control males, (2) our experimentally induced plasma T levels are similar to levels measured in free-living juvenile males of similar age, and (3) our demonstration of growth inhibition via exogenous T is corroborated by an increase in long-term growth in castrated males (with the caveat that testicular ablation does not exclusively test for the removal of T). Thus, all of our data support the hypothesis that T is a physiological growth inhibitor in *S. undulatus*, consistent with the prior demonstration of reduced mass gain and fat storage following T implantation in adult males of this species (Klukowski et al. 1998). While definitive experimental work remains to be done in order to identify the exact molecular, physiological, and behavioral mechanisms involved in T-mediated growth inhibition, our data present no reason to reject the prevailing hypothesis that T inhibits skeletal growth in juvenile squamate reptiles.

Contrary to our results, testosterone and other androgens are generally considered to be anabolic steroids that promote muscular and skeletal growth in other vertebrate classes. This generalization encompasses numerous male-larger mammals (reviewed in Ford and Klindt 1989), teleost fishes (reviewed in Holloway and Leatherland 1998), and birds (Fennel and Scanes 1992a; Schwabl 1996; but see Fennel and Scanes 1992b). However, castration apparently has no effect on male growth in one male-larger amphibian (Hayes and Licht 1992) and actually promotes male growth in one of the few female-larger mam-

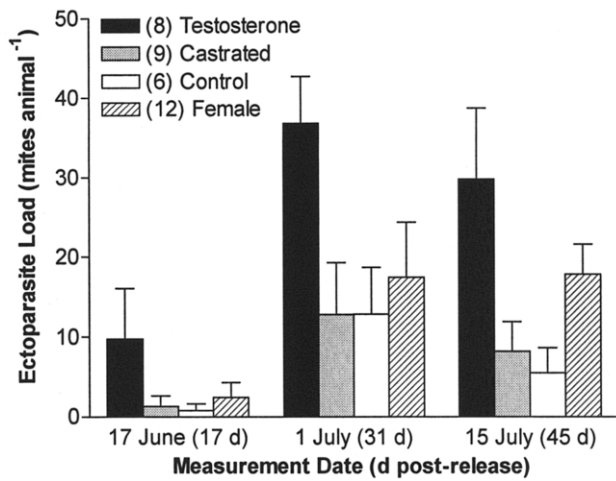


Figure 4. Mean (± 1 SE) ectoparasite load for treatment and sex groups at three recapture dates in 2002. Numbers in parentheses indicate sample sizes. Values were \log_{10} transformed before analysis. Repeated-measures ANOVA revealed a significant effect of time ($F_{2,40} = 15.22, P < 0.001$) and treatment ($F_{2,26} = 6.06, P = 0.009$) such that testosterone males had higher ectoparasite loads than castrated and control males. Repeated-measures ANOVA comparing control males and females revealed significant effects of time ($F_{2,32} = 35.34, P < 0.001$) and the sex-by-time interaction ($F_{2,32} = 3.37, P = 0.047$) but not sex ($F_{1,16} = 2.47, P = 0.136$). Because of the sex-by-time interaction, females had higher ectoparasite loads than control males at 45 d ($F_{1,16} = 10.05, P = 0.006$).

mals studied to date (Swanson 1967). Exceptions aside, the prevalent role of T as a male growth promoter in most vertebrate taxa stands in contrast to the emerging pattern of growth inhibition by T among squamate reptiles.

An understanding of the mechanistic basis of T-mediated growth regulation will almost certainly help to clarify such contrasts. One intriguing possibility is that growth promotion versus inhibition by T may occur via fundamentally distinct mechanisms. For example, growth-promoting effects of androgens in fishes are accomplished by increases in the transcription, synthesis, and secretion of mitogens such as growth hormone and insulin-like growth factor I (e.g., Holloway and Leatherland 1998; Riley et al. 2002). In similar fashion, androgens may interact with these endogenous growth regulators to directly mediate sexual differences in growth among male-larger mammals (Wehrenberg and Giustina 1992; Borski et al. 1996). Unfortunately, the cellular and molecular endocrinology of T-mediated growth inhibition has not been directly investigated among reptiles. However, from an organismal perspective, most studies of reptiles suggest that T inhibits growth indirectly by stimulating energetically expensive patterns of behavior, activity, and movement (see Marler and Moore 1989, 1991; Marler et al. 1995; Abell 1998; Klukowski et al. 1998; Salvador and Veiga 2000). We have proposed such a mechanism as an explanation for juvenile male growth inhibition and female-larger

SSD in *S. undulatus*. Having concluded that T inhibits juvenile male growth in this species, we now turn our attention to the central issue of our discussion: can this growth inhibition be explained by indirect, T-mediated energetic trade-offs?

Growth and Energetic Trade-Offs

In association with reduced growth rate, we observed substantial increases in daily activity period, daily movement, home range area, and ectoparasite load in castrated male *S. undulatus* treated with exogenous T. If any of these factors are involved in growth regulation, then they must alter the total amount of energy acquired by an animal (energy acquisition trade-off) and/or the fraction of assimilated energy allocated to growth (energy allocation trade-off). In free-living adult male *Sceloporus jarrovi*, exogenous T administration increases the frequency of territorial aggressive behaviors and reduces both the frequency of spontaneous feeding and the subsequent mass of gut contents (Marler and Moore 1988, 1989). Thus, a time allocation trade-off between aggression and feeding may force an energy acquisition trade-off that contributes to the reduction in body mass and stored energy observed among T-implanted males of this species (Marler and Moore 1989, 1991; see also Klukowski et al. 2001). Contrary to Marler and Moore’s (1989) results for *S. jarrovi* and despite the methodological similarity of our respective measures of spontaneous feeding, we did not see a reduction in food consumption following exogenous T administration in field-active *S. undulatus*. While our measures of food consumption are admittedly coarse, we nonetheless conclude that the growth effects of T in our experiments cannot be adequately explained by a reduction in energy acquisition.

Hormonal manipulations often alter patterns of energy allocation. At the most proximate physiological level, exogenous T may increase the oxygen consumption of specific tissues

Table 2: Food intake in *Sceloporus undulatus* sex and treatment groups

Treatment Group	Spontaneous Feeding (Meals h ⁻¹)		Laboratory Appetite (Crickets d ⁻¹)	
	N	Mean \pm 1 SE	N	Mean \pm 1 SE
Testosterone	7	1.19 \pm .43	8	2.75 \pm .29
Castrated	8	.75 \pm .32	9	2.44 \pm .31
Control	6	1.14 \pm .36	6	2.28 \pm .35
Female	12	3.14 \pm .42

Note. Spontaneous feeding was estimated from behavioral observations of undisturbed animals in the field enclosure in 2001. Laboratory appetite was measured in captivity under ad lib. food conditions at the conclusion of the 2002 experiment. ANOVA confirmed that male treatment had no effect on either spontaneous feeding ($F_{2,17} = 0.85, P = 0.445$) or laboratory appetite ($F_{2,20} = 0.54, P = 0.594$). Control males and females did not differ in laboratory appetite (ANOVA; $F_{1,16} = 1.78, P = 0.100$).

(Chandola et al. 1974a, 1974b; Thapliyal et al. 1974), thereby elevating whole-animal standard metabolic rate (SMR; Gupta and Thapliyal 1985; but see Marler et al. 1995). Similar effects on SMR have been reported for *S. undulatus* treated with exogenous thyroxine (T_4), which translates into a moderate elevation in total daily metabolic rate among field-active animals (Joos and John-Alder 1990). In the only study of its kind on lizards to date, Marler et al. (1995) found evidence for increased daily metabolic expenditure among field-active adult male *S. jarrovi* treated with exogenous T. Those authors attributed this result to the energetic cost of elevated territorial defense, which is similar to our hypothesized mechanism for T-mediated growth inhibition in *S. undulatus*.

To evaluate the potential for T-mediated energy allocation trade-offs with growth, we reviewed the relevant literature concerning energetic costs of growth, movement, daily activity, and parasitism. When possible, we used published estimates of such costs to make hypothetical calculations comparing the relative magnitude of energy invested in growth versus these other allocation sinks. For simplicity, we expressed all mass-specific literature estimates of energetic cost as the hypothetical equivalent for a 3.5-g lizard, which was the overall mean size of males in our experiments.

Energetic Cost of Growth. On average, control males grew 19.39 mg d^{-1} more than testosterone males. Assuming a whole-animal tissue energy content of about 4.84 J mg^{-1} wet mass (derived from Nagy 1983; Angilletta 1999), we estimated that the production cost of growth (i.e., the energy deposited in new tissue) is about 94 J d^{-1} greater in control than testosterone males. Literature estimates of the additional respiratory cost of growth (i.e., the metabolic cost of tissue biosynthesis) are quite variable, but Peterson et al. (1999) derived a robust estimate of 0.38 J metabolic expenditure per joule deposited in new tissue among free-living garter snakes (*Thamnophis sirtalis*). When applied to our data and combined with production costs, this value yields a total energetic growth cost (production + respiration) that is 130 J d^{-1} greater for control than testosterone males. Can this difference in energy allocated to growth be explained by associated differences in the energetic costs of movement, daily activity period, and parasitism?

Energetic Cost of Movement. On average, testosterone males moved a minimum of 30 m d^{-1} further than control males (Fig. 3), and our measure of estimated daily movement suggests that the actual magnitude of this difference may be closer to 107 m d^{-1} . On the basis of the allometric equation derived from 19 lizard species by John-Alder et al. (1986), the estimated net cost of transport for a 3.5-g lizard is 208 J km^{-1} . This amounts to an approximate difference in energy expenditure of 6–22 J d^{-1} between testosterone and control males over our range of daily movement estimates. Thus, only about 5%–17%

of our estimated 130-J d^{-1} difference in energy allocated to growth can be explained by the cost of movement.

Energetic Cost of Activity. Even when an ectothermic animal is sedentary, it incurs an energetic cost of activity when it maintains a T_b above that which it would experience while inactive (i.e., in a burrow, crevice, etc.). This cost is imposed by the thermal increment (Q_{10}) of metabolic rate. To derive the thermal increment in resting metabolism for active animals, we used Angilletta's (2001) regression models predicting the summer resting metabolic rate (RMR, metabolic energy expenditure of an inactive, postabsorptive animal at a given T_b during photophase) of New Jersey *S. undulatus*. By assuming equivalence between our treatment groups for active T_b (33°C; see "Body Temperature" in the "Results" section) and inactive T_b (20°C; S. Skelly, unpublished data), we estimated that the thermal increment in RMR imposes an approximate cost of 5.89 J $g^{-1} h^{-1}$ of activity. On average, the daily activity period of our testosterone males exceeded that of control males by 1.5 h d^{-1} (Fig. 3A). Hence, the estimated cost of this increase in daily activity period for a 3.5-g *S. undulatus* is about 31 J d^{-1} , which is comparable to values predicted for other *Sceloporus* species (Fig. 5). Thus, the thermal increment in RMR can account only for 24% of our estimated 130-J d^{-1} difference in energy allocated to growth between testosterone and control males.

In addition to the thermal increment in RMR, activity metabolism must support social behavior, locomotion (see "Energetic Cost of Movement"), postural adjustments, foraging and prey capture, digestion, alertness and predator avoidance, and so forth. Not surprisingly, this "cost of free existence" (sensu Bennett and Nagy 1977) above resting expenditure typically exceeds the cost imposed by the thermal increment in RMR by a factor of two to four in *Sceloporus* lizards (Fig. 5). Using the allometric equation reported by Joos and John-Alder (1990) for adult *S. undulatus*, we estimated the total field metabolic rate for an unmanipulated, 3.5-g juvenile as about 183 J $g^{-1} d^{-1}$. By assuming an 8.5-h daily activity period (Fig. 3B, control males) and subtracting out total daily maintenance metabolism, we estimated the cost of free existence to be 13.48 J $g^{-1} h^{-1}$. Hence, for a 3.5-g *S. undulatus*, the additional cost of free existence accruing from a 1.5-h increase in daily activity period is about 71 J, or roughly 55% of our estimated 130-J d^{-1} difference in energy allocated to growth. This value is within the typical range of 48–159 J (37%–122%) estimated across other *Sceloporus* species, which reflects additional variance among environments, seasons, sexes, and age classes (Fig. 5). It should be noted that the cost of free existence, calculated in this fashion, also includes the metabolic cost of growth. However, in most cases, growth is not likely to comprise a substantial fraction of our estimated costs of free existence (Fig. 5) because these field metabolic rate data were typically obtained from adult lizards exhibiting little or no growth.

Combining the cost accruing from thermal increment in

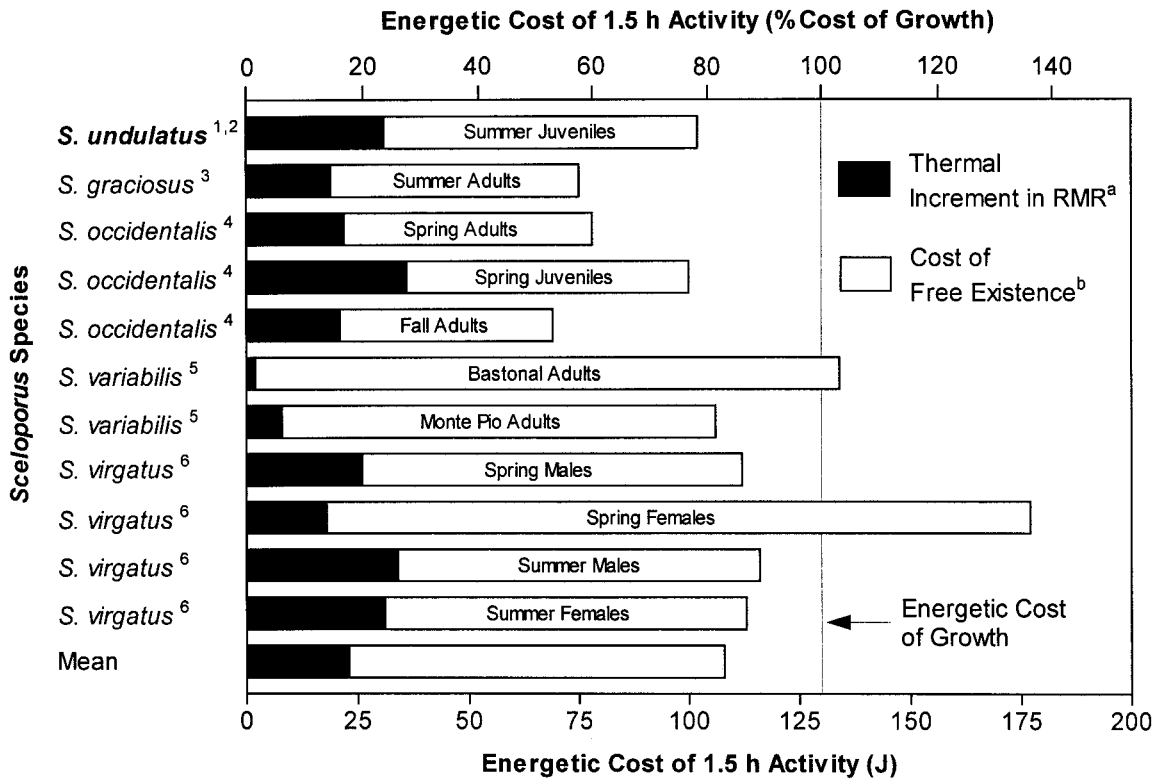


Figure 5. Hypothetical energetic cost of 1.5 h activity (difference in daily activity period between testosterone and control male *Sceloporus undulatus*) for a 3.5-g lizard (mean size of experimental male *S. undulatus*), estimated for several *Sceloporus* species based on published time/energy budgets. Costs are expressed in absolute joules (*lower axis*) and as percentages (*upper axis*) of our estimated 130-J d⁻¹ difference (*vertical line*) in energy allocated to growth between testosterone and control male *S. undulatus*. See text (“Energetic Cost of Growth”) for our derivation of this energetic cost of growth. Across species, the total cost of activity accounts for 53%–137% (mean 83%) of the cost of growth. RMR = resting metabolic rate, metabolic expenditure while inactive and postabsorptive at a given T_b ; FMR = field metabolic rate, total daily metabolic expenditure, estimated via doubly labeled water in field-active animals; T_b = body temperature, estimated for inactive animals in burrows and active animals while abroad. $a = (\text{RMR at active } T_b) - (\text{RMR at inactive } T_b)$. $b = \text{FMR} - (\text{RMR at active } T_b \times \text{active period}) - (\text{RMR at inactive } T_b \times \text{inactive period})$. 1 = RMR estimated from equations of Angilletta (2001, Table 3). 2 = FMR estimated from equation of Joos and John-Alder (1990). 3 = Data from Congdon and Tinkle (1982, Table 2). 4 = Data from Bennett and Nagy (1977, Table 2). 5 = Data from Benabib and Congdon (1992, Table 2). 6 = Data from Merker and Nagy (1984, Table 3).

RMR with the additional cost of free existence, we derived an estimated total daily cost of 102 J for a 1.5-h increase in daily activity period by a 3.5-g *S. undulatus*. This estimate is similar to the mean cost of a hypothetical 1.5-h increase in daily activity period calculated across *Sceloporus* species (108 J; Fig. 5). This 102-J d⁻¹ cost of increased daily activity period represents about 79% of our estimated 130-J d⁻¹ difference in energy allocated to growth between testosterone and control male *S. undulatus*, and the broader range of hypothetical values across *Sceloporus* species covers 53%–137% of this difference (Fig. 5). Thus, we conclude that the energetic cost of increased daily activity period is of sufficient magnitude to explain much of our observed effect of T on growth.

For several reasons, our calculations might actually underestimate the true magnitude of the cost of increased daily activity period in testosterone males. First, our measures of daily

activity period were obtained primarily under optimal weather conditions and never during inclement weather (i.e., cold, rain). In subsequent experiments, we have frequently found T-implanted males active in suboptimal weather, at times when control males and females were generally inactive (R. Cox and H. John-Alder, personal observations). This agrees with our observation that testosterone males in this study were active both early and late in the day (i.e., in presumably suboptimal thermal conditions) and suggests that treatment effects on daily activity period might be even more pronounced under suboptimal weather conditions. Additionally, our estimates of treatment differences in the cost of free existence assume equivalence in the intensity of activity among treatments. This is clearly not the case because we observed an approximately two-fold difference in spontaneous movement (m h⁻¹) between testosterone and control males. Interestingly, Merker and Nagy

(1984) attributed a similar twofold difference in cost of free existence between adult male and female *Sceloporus virgatus* (Fig. 5, spring) to underlying differences in the intensity of activity while abroad.

Energetic Cost of Parasitism. Overall, mite loads were lowest in mid-June and highest in early July, presumably reflecting seasonal changes in mite abundance (Klukowski 2004). On average, we observed about 18 more mites per animal on testosterone than on control males, an approximately fourfold difference in ectoparasite load (Fig. 4). Similar increases in parasite load following T administration have been documented in this and other lizard species (Salvador et al. 1996; Olsson et al. 2000; Klukowski and Nelson 2001). Parasitism may impose a substantial energetic cost on lizards, as suggested by associated reductions in body mass, fat storage, tail regeneration, and survival (Schall 1983; Sorci and Clobert 1995; Salvador et al. 1996; Sorci et al. 1996; Oppliger and Clobert 1997). Such costs may also inhibit growth, as evidenced by the 4% reduction in growth among *Sceloporus occidentalis* infected with the malarial blood parasite *Plasmodium mexicanum* (Schall 1983). Further, energetic trade-offs between parasitism and growth may be accentuated by an interaction with T. For example, juvenile *Lacerta vivipara* lizards that have been prenatally exposed to experimentally elevated T levels exhibit reduced postnatal mass gain following tick infestation, but this effect of parasitism is not observed in the absence of embryonic T manipulation (Uller and Olsson 2003). Similar interactions might explain why our experimental females grew significantly faster than control males (Fig. 2B) despite relatively higher ectoparasite loads (Fig. 4).

Natural Significance of Experimental Results

Exogenous T clearly inhibits growth and increases daily activity period, daily movement, home range area, and ectoparasite load in juvenile male *S. undulatus*, and relationships among these responses suggest an energetic trade-off at the expense of growth. However, plasma T, daily activity period, and daily movement did not differ between control and castrated males during our experiments, and the higher long-term growth rate of castrated males was apparent only at 418 d postrelease. Further, plasma T, daily activity period, daily movement, and home range area did not differ significantly between control males and females despite a marginally significant sex difference in growth rate. These inconsistencies require that we critically analyze our results to determine their significance with regard to natural patterns of plasma T, behavior, and growth.

During the period in which we conducted our experiments (June–August), mean plasma T in free-living juvenile males was substantially higher than in free-living females (Fig. 1, July). We were therefore surprised that we did not detect differences in plasma T between control males and either castrated males

or females at 45 d postrelease (late July). Although individual free-living juvenile males with low plasma T are not uncommon at this time of year, the uniformly low plasma T exhibited by our control males is not representative of the overall mean in free-living males. In fact, the difference in plasma T between free-living juvenile males and females is much closer in magnitude to the difference observed between experimental testosterone males (mean plasma T similar to free-living males) and all other treatment groups (mean plasma T similar to free-living females) than to the insignificant difference between control males and either castrated males or females. We can only speculate as to the cause of the atypically low plasma T observed in control males, but it is conceivable that our experimental conditions (i.e., removal from natural home range, enclosure confinement, artificial density of juvenile males) may have introduced a caging artifact by suppressing plasma T. While low plasma T in control males at 45 d postrelease was surprising, low plasma T in this same group at 418 d postrelease (late July of the subsequent year) is consistent with plasma T in free-living adult males. Maximal plasma T levels typically occur in adult males at the onset of the breeding season (mean \pm 1 SE = 52.80 ± 5.24 ng mL⁻¹ in March), after which plasma T declines to basal levels (<2 ng mL⁻¹ in July) in most individuals (H. John-Alder, G. Haenel, and L. Smith, unpublished data). Presumably, plasma T was elevated in our experimental control males at the end of the activity season during which experiments were conducted and/or during the following breeding season (between our 45- and 418-d samples). Size divergence between control and castrated males may have occurred primarily during these periods.

Given that control males were not representative of natural juvenile males with regard to plasma T, they may have been similarly atypical with regard to behavior (e.g., daily activity period, movement). Although we have previously documented sex differences in aggression and movement during the period of sexual growth divergence (Skelly and John-Alder 2002), we did not observe significant differences in daily activity period, daily movement, or home range area between control males and females. These results contradict our hypothesis that sex differences in growth accrue from underlying differences in these targets of energy allocation, especially in light of the difference in growth rate observed between control males and females. However, the effect of sex on growth rate was relatively weak in our experiment ($P = 0.04$, one-tailed) such that mean female growth rate exceeded that of control males by only 19% as compared to ca. 42% between natural juvenile males and females during a comparable period (calculated from data in Haenel and John-Alder 2002). Thus, inconsistencies between our data and our predictions may be due in part to unrepresentative patterns of plasma T, behavior, and growth in control males.

Even though plasma T did not differ among control males, castrated males, and females, it must be emphasized that phys-

iological responses to T depend on the hormone's physiological activity (e.g., free vs. bound hormone, receptor saturation), not simply its total plasma concentration. Just as significant seasonal variation in plasma hormone concentration does not obligate any particular physiological response, the absence of variation in plasma hormone concentration does not preclude variation in physiological activity. Indeed, measurements of total plasma hormone levels can be misleading in the absence of information on other factors that influence physiological activity (e.g., binding globulins; Lynn et al. 2003). In our experiments, control males developed bright ventral coloration, while castrated males and females did not, strongly suggesting that the physiological activity of T differed among these groups despite their overall similarity in plasma T.

Implications for SSD

Studies of SSD have traditionally focused on differences in the selective pressures acting on male versus female body size (e.g., Cox et al. 2003). However, relatively little attention has been directed toward the possibility that SSD may often arise indirectly, from sexual differences in growth constraints accruing from selection on energetically expensive patterns of activity, behavior, or reproductive investment. Here, we have presented evidence in support of the hypothesis that female-larger SSD in *S. undulatus* arises as a result of energetic costs of maturational increases in daily activity period and movement in juvenile males. Opposite patterns of male-larger SSD may often result from the preferential allocation of energy to the production of offspring rather than growth in sexually mature females (e.g., Sugg et al. 1995). In lizards, energetic costs of reproduction for both males and females have been inferred from descriptive quantifications of metabolic expenditure in both the field (e.g., Nagy 1983; Merker and Nagy 1984) and lab (e.g., Beuchat and Vleck 1990; Angilletta and Sears 2000), as well as from experimental manipulations of reproductive investment (e.g., Landwer 1994; Marler et al. 1995). However, such studies have yet to be rigorously integrated within the theoretical framework of SSD. Furthermore, even when sexual differences in body size are presumably adaptive in their own right, we lack a fundamental understanding of the physiological mechanisms (e.g., sex steroids) whereby sexual differences in growth are mediated so as to minimize intersexual genetic conflict (see Badyaev 2002). Thus, additional studies of the proximate behavioral and physiological mechanisms involved in growth regulation should greatly improve our understanding of SSD.

Acknowledgments

The foundations for this study were laid during prior collaboration with Greg Haenel. We are grateful to Laura Branagan,

Russell Duncan, Robert Hopkins, and Kristin Mylecraine for field assistance and help constructing the experimental enclosure. We thank John Dighton and the staff of the Rutgers University Pinelands Research Station for logistical support and the use of land, laboratory, and housing facilities during this study. This project was funded by National Science Foundation grant IBN-0135167 to H.B.J.-A.

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