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Environmental sensitivity of sexual size dimorphism: laboratory common garden removes effects of sex and castration on lizard growth

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Summary

1. Adult males average 10% larger than females in natural populations of Yarrow's Spiny Lizard (*Sceloporus jarrovii*). In two previous studies of free-living animals, we found that (1) this sexual size dimorphism (SSD) develops because yearling males grow more quickly than females and (2) the sex steroid testosterone (T) may regulate this sex difference in growth: castrated males (CAST) grow more slowly than either intact control males (CON) or castrated males treated with exogenous T (TEST).
2. In the present study, we tested the environmental sensitivity of these sex and treatment effects on growth by raising captive males (CAST, CON, TEST) and females under identical 'common garden' conditions.
3. Sex and treatment effects on growth rate were absent in captivity. The development of SSD was suppressed because captive males grew more slowly than free-living males of equal size, while experimental treatments failed to affect male growth because CAST grew more quickly in the laboratory common garden than in the field.
4. Individual growth rates were strongly related to food consumption, but feeding rate did not differ between sexes or among male treatments.
5. Our results call attention to the environmental sensitivity of sex-specific endocrine growth regulation and illustrate the importance of combining laboratory and field studies of growth and SSD.

Key-words: Body size, growth rate, proximate mechanism, testosterone

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Introduction

Sexual size dimorphism (SSD), defined as a difference in the body size of conspecific males and females, is a ubiquitous biological phenomenon. Darwin (1871) attributed such dimorphisms to sex differences in the selective forces acting on body size, and more than a century of subsequent research has focused primarily on the evolutionary causes of SSD (reviewed in Andersson 1994). By contrast, relatively little is known about the underlying physiological mechanisms that mediate sex differences in growth (Duvall & Beaupre 1998; Badyaev 2002; Cox & John-Alder 2005; Cox *et al*. 2005a). In the present study, we ask (1) are natural sex differences in growth influenced by proximate environmental factors and (2) are underlying endocrine mechanisms for sex-specific growth regulation similarly dependent upon environmental context?

Yarrow's Spiny Lizard (*Sceloporus jarrovii* Cope) exhibits pronounced SSD, such that adult males average 10% larger (i.e. longer in snout–vent length) than adult females (Fitch 1978; Ruby & Dunham 1984). This dimorphism develops during the first year of life because yearling males grow more quickly than females (Cox 2005). In other species, natural sex differences in growth are often absent under controlled laboratory conditions. For example, female-larger SSD develops in free-living *Sceloporus undulatus* lizards because females grow more quickly than males, but sex differences in size and growth are absent in captivity (Haenel & John-Alder 2002). Similarly, female-larger SSD develops in free-living *Eleutherodactylus coqui* frogs because males stop growing upon maturation, but captive males grow quickly and attain large body sizes characteristic of females (Woolbright 1989). Although *Crotalus atrox* rattlesnakes exhibit male-larger SSD in the wild, captive females equal males in growth rate and even exceed males in body mass when both sexes are raised on high-intake diets (Taylor &

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DeNardo 2005). These findings suggest that males and females may often share similar genetic growth potentials, but express different growth phenotypes because of inherent ecological differences (e.g. time and energy budgets, thermal microhabitats, foraging niches). In the present study, we test the environmental sensitivity of natural sex differences in growth by raising captive *S. jarrovii* males and females under identical 'common garden' laboratory conditions (i.e. *ad libitum* food, social isolation, controlled thermal environment and photoperiod).

To minimize intersexual genetic conflict, sex differences in growth are predicted to involve epigenetic interactions with sex-specific growth regulators (Badyaev 2002). Recently, we have shown that the sex steroid testosterone (T) may act as a bipotential growth regulator by inhibiting growth in species with female-larger SSD and stimulating growth in species with male-larger SSD (Cox & John-Alder 2005; Cox *et al*. 2005a). Specifically, we found that castration can stimulate male growth in *Sceloporus* species with female-larger SSD, whereas treatment with exogenous T inhibits male growth in such species. By contrast, castration inhibits the growth of free-living *S. jarrovii* males, while exogenous T restores growth of castrated males to the rate of intact controls. In the present study, we test the environmental sensitivity of this growth response to castration and exogenous T by manipulating captive *S. jarrovii* males in a common garden laboratory environment.

Materials and methods

ANIMAL COLLECTION AND EXPERIMENTAL DESIGN

We collected *Sceloporus jarrovii* yearlings near Buena Vista Peak in the Chiricahua Mountains, Coronado National Forest, Arizona, USA (31°54–55′N, 109°16′W). We determined sex by the presence (males) or absence (females) of enlarged postanal scales and collected 32 males and 13 females in September 2003, and 29 males in September 2004, In September, yearlings (age *c*. 3 months, born in June) can be reliably distinguished from older animals on the basis of size. Further, sex differences in growth are pronounced at this time, making the timing of our experiments appropriate with respect to the natural ontogeny of SSD (Cox 2005).

Animals were transported to Rutgers University and housed individually in plastic cages ($36 \times 42 \times 46$ cm^3) containing sand bedding and two bricks that were stacked to form a shelter and basking site. Water was always available in a shallow dish lined with aquarium gravel. We provided heat by suspending an incandescent spotlight (Philips 65 W BR-40SP, Royal Philips Electronics, Netherlands) above each basking site. Cages were arranged under a bank of fluorescent bulbs (General Electric Chroma 50, General Electric Company, Fairfield, CT, USA) for ultraviolet (UV) radiation. Cages did not have lids and perches were located within 0·3 m of the fluorescent bulbs to maximize UV exposure, which is important for calcium metabolism and hence lizard growth. Fluorescent lights were set on timers to provide a daily 12:12 h light : dark photoperiod and spotlights were set to provide a 10-h basking period.

We measured snout–vent length (SVL) to the nearest 1 mm with a ruler and body mass to the nearest 0·02 g with an electronic balance. Males were then assigned to one of three size-matched treatment groups: bilaterally castrated males receiving a T implant (TEST); bilaterally castrated males receiving a placebo implant (CAST); and intact, control males receiving a sham surgery and placebo implant (CON). Females received a sham surgery and placebo implant to facilitate direct comparisons with CON males. Testosterone implants were constructed of Silastic® tubing (Dow Corning, Midland, MI, USA, 1.47 mm i.d., 1.96 mm o.d.) and contained 300 µg of crystalline T (Sigma T-1500, Sigma-Aldrich Inc., St. Louis, MO, USA) within a 1·5 mm lumen sealed by silicone adhesive. See Cox & John-Alder (2005) for a detailed description of surgical procedures and implant construction. Survival following surgery was high in both 2003 (42 of 45, 93%) and 2004 (28 of 29, 97%). Following surgery, we measured SVL and body mass at biweekly intervals over the course of a 49-day (2003) or 42-day (2004) experimental period. For sex comparisons, we continued to measure size at biweekly intervals through 260 days (37 weeks) post-treatment.

MALE TREATMENT VALIDATIONS

To determine whether our treatments produced their intended physiological effects, we collected blood samples from each animal at 42 days post-treatment in the 2004 experiment. Samples were processed and assayed for plasma T using radioimmunoassay (RIA) methods reported elsewhere (Cox & John-Alder 2005; Cox *et al*. 2005a). The limit of detection for the present assay was 4·2 pg T. We did not sample blood in the 2003 experiment, but we digitally scanned the ventral surface of each animal at 49 days post-treatment to measure two secondary sexual traits that are influenced by T: blue throat coloration (see Cox *et al*. 2005b) and basal tail width (reflecting enlargement of the hemipenes). Digital images were obtained using an Epson® Perfection® 1240 U scanner and analysed using Adobe® PhotoShop® software (version 7·0·1). We used the method of Cox *et al*. (2005b) to estimate hue (measured on a standard 360° colour wheel), saturation (0% = grey, 100% = fully saturated) and brightness (0% = black, 100% = white) from these images. We measured tail width just caudal to the vent by standardizing actual measurements of each image against the digital image of a ruler that was scanned alongside each animal.

GROWTH RATE

Following convention in the reptile literature, we focus our analyses of growth and SSD on length, although

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we do briefly mention sex and treatment effects on body mass when appropriate. Increases in SVL were linear over the 49-day and 42-day measurement periods of our T manipulation experiments, so we calculated growth rate as the slope of the linear regression of SVL against elapsed time for each animal. However, growth rates were non-linear over the duration of our 37-week sex comparison, so we calculated separate linear growth rates for each 4-week interval by subtracting initial SVL from final SVL and dividing by elapsed time. We calculated rate of change in body mass over each interval in analogous fashion. To compare growth rates between captive and free-living animals, we used data from an extensive mark–recapture study of the population from which we collected yearlings. These mark–recapture data are reported and analysed elsewhere, and are presented here only when appropriate for comparison with laboratory results. We restricted all comparisons to free-living animals of the same age and size range as captive animals.

FOOD CONSUMPTION AND FAT STORAGE

Throughout our experiments, we offered each lizard three small crickets (*Acheta domestica*, 2–4 weeks old, 6–12 mm length) per day for food. Crickets were fed mixed hay and were found to contain 78% crude protein, 11% crude fat and 11% ash by dry mass (Dairy One, Inc., Ithaca, NY; R. M. Cox, M. M. Barrett & H. B. John-Alder, unpublished data). Crickets were occasionally dusted with Fluker's 2:1 calcium : phosphorus dietary supplement. To examine sex and treatment effects on energy intake, we searched each cage every 3 days and counted and removed all remaining crickets. We then subtracted the number of crickets remaining from the number offered to estimate the number consumed during the interval. We measured food consumption over the entire 42-day experimental period in 2004, but only during two representative 2-week periods during the 2003 study. To investigate the possibility of treatment differences in energy allocation to storage, we dissected experimental males and measured the wet mass of their abdominal fat bodies at the conclusion of the 2004 study.

STATISTICAL ANALYSES

All statistical analyses were implemented with SAS (version 8·2, SAS Institute Inc., Cary, NC). Sex differences were inferred from comparisons of sham-operated (CON) males and females. We tested for sex differences in growth over time using repeated measures ANOVA with sex as a between-subjects effect, time as a within-subjects effect and a sex-by-time interaction term. We evaluated sex and sex-by-time effects using multivariate Wilks' lambda tests. To account for the fact that males were initially larger than females and growth rate decreases with body size, we also analysed growth rates for SVL and mass separately for each interval using ANCOVA with sex as the main effect and SVL or mass at the beginning of each interval as a covariate. For each interval, we verified that allometric slopes did not differ between sexes prior to ANCOVA.

Within each T manipulation experiment (2003 and 2004), we tested for treatment effects using ANCOVA with male treatment (CAST, CON, TEST) as the main effect and SVL or body mass as a covariate. We verified the assumption of homogeneous slopes among treatment groups prior to ANCOVA. When size covariates were not necessary, we used ANOVA with treatment as the main effect. To pool data from both experiments, we included year as a categorical effect in our statistical models. We never observed significant interactions between year and either treatment or the size covariate, so we did not include these interaction terms in our final statistical models.

To examine intra-individual correlations between growth rate and feeding rate, we calculated residuals from the least-squares linear regression of each measure on SVL. We then used these 'size-corrected' residuals to test for relationships between growth and feeding. We generated residuals using separate regressions for each year and treated sex and treatment groups as equivalent because previous analyses found no effect of sex or treatment on either growth rate or feeding rate (see below).

Results

SEX EFFECTS

Males averaged 2·4 mm (4·5%) larger than females when we collected them for our experiments. However, using repeated measures ANOVA, we found no overall sex difference in subsequent growth rate over the duration of our 37-week experiment ($F_{1,21}$ = 0·66; P = 0·426; Fig. 1). This analysis revealed a significant effect of time on growth ($F_{7,15}$ = 13·91; P < 0·001), but no sex-by-time interaction ($F_{7,15}$ = 1·12; P = 0·399), such that male and female growth rates decreased similarly with time (Fig. 1). We obtained similar results for the rate of change in body

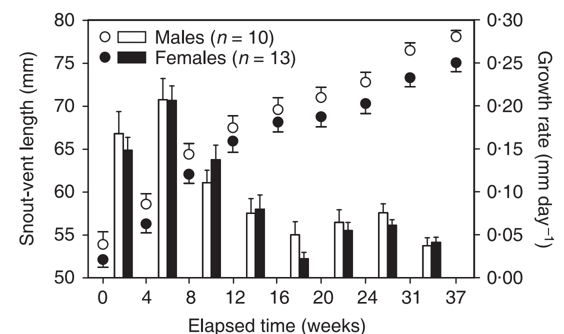

Fig. 1. Mean (± 1 SE) snout–vent length (circles) and growth rate (bars) *vs* elapsed time for males and females raised in captivity for 37 weeks. Linear growth rates are calculated separately for each interval because repeated measures ANOVA revealed a significant effect of time on growth rate. For clarity, size is plotted at intervals of 4 weeks or longer, although biweekly size measurements were collected. Effects of sex and the sex-by-time interaction were not significant (see text and Table 1 for statistical results).

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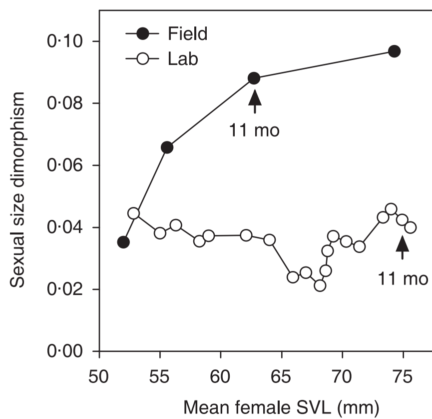

Fig. 2. Development of sexual size dimorphism (SSD) as a function of mean female snout–vent-length (SVL) in captive (lab) and free-living (field) yearlings. SSD is calculated as: (mean male SVL/mean female SVL) – 1. SSD is expressed as a function of mean female SVL to facilitate direct comparisons between field and lab data. Arrows indicate measurements for animals at about 11 months of age. The development of SSD is suppressed in captivity, regardless of whether field and lab animals are compared on the basis of age or mean female size.

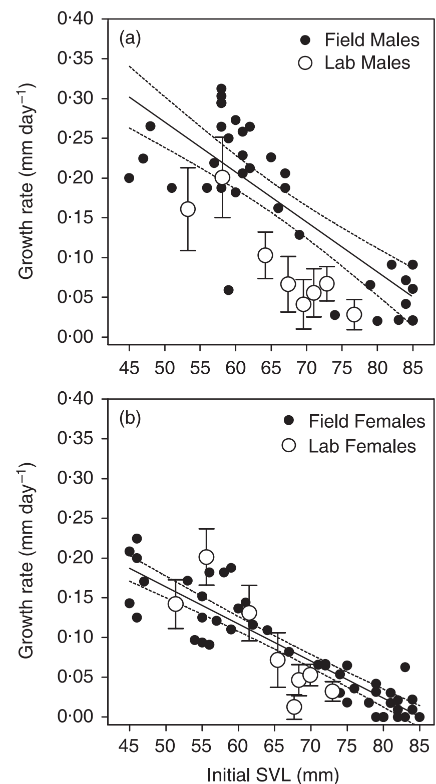

Fig. 3. Comparison of size-specific growth rates for captive (lab) and free-living (field) yearling males (a) and females (b). Each free-living animal is represented by a single growth measurement, calculated over intervals of 3–5 weeks for yearlings within the same size range as captive animals. Regression line (±95% confidence interval) is derived from least-squares linear regression to illustrate typical size-dependence of growth in the field. Each data point for captive yearlings corresponds to the mean growth rate (± 2 SE) and SVL for successive monthly growth intervals in the lab study (see Fig. 1). Captive females grew at rates comparable to free-living females of similar size, while captive males grew more slowly than free-living males of comparable size.

mass, which decreased with time ($F_{7,15}$ = 12·83; P < 0·001) but was not related to sex ($F_{7,15}$ = 1·57; P = 0·223) or the sex-by-time interaction ($F_{7,15}$ = 1·54; P = 0·154). Because growth rate decreased with size and males were slightly larger than females, we also examined SVL growth rates separately over each interval using ANCOVA with SVL as a covariate. With one exception, we found no relationship between SVL and growth rate, and sex differences in growth were generally absent, regardless of whether or not we included the size covariate (Table 1). Results were qualitatively identical when we compared change in mass (data not shown). We did not detect a sex difference in feeding rate at either 3–5 weeks ($F_{2,20}$ = 0·71; P = 0·408) or 13–15 weeks ($F_{2,20}$ = 0·46; P = 0·504) post-treatment.

After 37 weeks in captivity, males had attained a mean size of 78·6 mm SVL, only 3·0 mm (4·0%) larger than the mean female SVL of 75·6 mm. Thus, the magnitude of sexual size dimorphism remained essentially unchanged from the slight (2·4 mm) sex difference in size at the start of the experiment (Fig. 2). In contrast, by the time that free-living females attain a size comparable to that of the captive females, males average about 7·2 mm (9·7%) larger (Fig. 2). To determine whether this suppression of SSD in captivity resulted from an increase in female growth rate or a decrease in male growth rate, we compared growth rates between captive and free-living animals. For any given size, free-living males grew more quickly than captive males, but growth rates of captive and free-living females were generally similar (Fig. 3).

Table 1. Statistical results for comparisons of CON male and female growth rate over successive time intervals. Results are reported for ANCOVA with sex as a categorical effect and snout–vent length (SVL) as a covariate. Sex effects on growth are weak (P > 0·02) or absent (P > 0·05), regardless of whether SVL is included (Type III sum-of-squares) or excluded (Type I sum-of-squares) as a covariate

Interval (week)	df	Sex (Type I SS)		Sex (Type III SS)		SVL (covariate)	
		F	P	F	P	F	P
0–4	2,20	0·50	0·489	0·97	0·336	1·75	0·201
4–8	2,20	0·00	0·970	0·11	0·738	1·04	0·319
8–12	2,20	1·32	0·265	2·00	0·172	1·17	0·293
12–16	2,20	0·04	0·846	0·00	0·949	2·09	0·163
16–20	2,20	3·41	0·080	4·49	0·047	3·03	0·097
20–24	2,20	0·30	0·592	0·54	0·469	0·60	0·447
24–31	2,19	2·16	0·158	5·89	0·025	11·21	0·003
31–37	2,19	0·14	0·711	0·05	0·822	1·64	0·215

MALE TREATMENT VALIDATIONS

At 42-days post-treatment, plasma T levels were uniformly low in CAST, intermediate and variable in CON, and elevated in TEST (Table 2). One TEST male had an atypically low plasma T concentration (0·70 ng ml^{-1}), presumably indicating that his implant had recently exhausted. However, plasma T levels differed significantly among all treatment groups, regardless of whether this

Table 2. Response variables used to verify physiological effects of castration and T implants. Lower-case italic letters denote statistical separation of treatment groups based on ANOVA with REGWQ *post-hoc* test (plasma [T], hue, saturation and brightness) or pairwise comparisons of least-square means derived from ANCOVA with SVL as a covariate (tail width). For all variables except throat brightness, CAST and TEST are statistically distinct, with CON exhibiting intermediate values

Response variable	Treatment	*N*	Mean ± 1 SE	
Plasma [T] (ng/ml)	CAST	10	0·80 ± 0·24	*a*
	CON	9	19·73 ± 6·89	*b*
	TEST	8	39·04 ± 7·61	*c*
Throat hue (degrees)	CAST	10	214·40 ± 1·81	*a*
	CON	10	219·70 ± 0·94	*b*
	TEST	9	220·00 ± 1·15	*b*
Throat saturation (%)	CAST	10	44·80 ± 4·39	*a*
	CON	10	52·20 ± 3·86	*a,b*
	TEST	9	63·89 ± 4·37	*b*
Throat brightness (%)	CAST	10	67·10 ± 2·59	*a*
	CON	10	62·00 ± 1·39	*a*
	TEST	9	63·89 ± 2·36	*a*
Tail width (mm)	CAST	10	11·55 ± 0·22	*a*
	CON	10	11·75 ± 0·40	*a*
	TEST	9	12·56 ± 0·26	*b*

animal was included ($F_{2,24}$ = 9·13; P = 0·001) or excluded ($F_{2,23}$ = 12·99; P < 0·001). Testosterone enhanced the intensity and richness of blue throat coloration (Table 2), such that treatment groups differed in hue ($F_{2,26}$ = 5·37; P = 0·011) and saturation ($F_{2,26}$ = 5·09; P = 0·014), but not brightness ($F_{2,26}$ = 1·46; P = 0·250). Tail width was positively related to SVL ($F_{3,25}$ = 19·52; P < 0·001), and TEST had significantly wider tail bases than either CAST or CON for any given SVL ($F_{3,23}$ = 5·60; P < 0·001; Table 2).

MALE TREATMENT EFFECTS

Male growth rate scaled negatively with initial SVL in 2003 ($F_{3,25}$ = 11·23; P < 0·003), but not in 2004 ($F_{3,24}$ = 0·04; P = 0·841). For consistency, we analysed both years using ANCOVA with SVL as a covariate, although treatment effects were identical without this covariate. Growth rate did not differ among treatments in either 2003 ($F_{3,25}$ = 0·70; P = 0·508; Fig. 4) or 2004 ($F_{3,24}$ = 0·34; P = 0·716; Fig. 4). Change in body mass also did not differ among treatments in either 2003 ($F_{3,25}$ = 2·41; P = 0·111) or 2004 ($F_{3,24}$ = 0·65; P = 0·530). Treatment effects on SVL growth rate were still not evident when we pooled data from both years ($F_{4,52}$ = 1·15; P = 0·326), but this analysis revealed a difference in growth between years ($F_{4,52}$ = 12·54; P < 0·001). Averaged across treatments, males grew 0·04 mm day^{-1} more quickly in 2003 than in 2004 (Fig. 4).

Averaged across years and treatments, experimental males consumed 2·31 crickets per day (minimum 1·36; maximum 2·86). Given this mean value and the fact that no individual ever achieved a consumption rate equal to food availability (three crickets day^{-1}), we consider food availability to be effectively *ad libitum* for these experiments. Feeding rate was not related to SVL in 2003 ($F_{3,25}$ = 0·41; P = 0·529), but feeding rate scaled positively with SVL in 2004 ($F_{3,24}$ = 7·20; P = 0·013). For consistency, we analysed both years using ANCOVA with SVL as a covariate, although treatment effects were identical without this covariate. We did not find any differences in feeding rate among treatments in either 2003 ($F_{3,25}$ = 2·38; P = 0·113; Fig. 5) or 2004 ($F_{3,24}$ = 0·22; P = 0·802; Fig. 5). Treatment effects on feeding rate were still not evident when we pooled data from both years ($F_{4,52}$ = 1·85; P = 0·168), but feeding rates were slightly different between years ($F_{4,52}$ = 4·63; P = 0·036). Averaged across groups, males ate 0·2 more crickets per day in 2004 than in 2003 (Fig. 5). Wet mass of abdominal fat bodies was positively correlated with body mass ($F_{3,21}$ = 41·88; P < 0·001; both variables $\log_{10}$-transformed), but we did not detect any treatment effect on this measure of energy storage ($F_{3,21}$ = 0·56; P = 0·578).

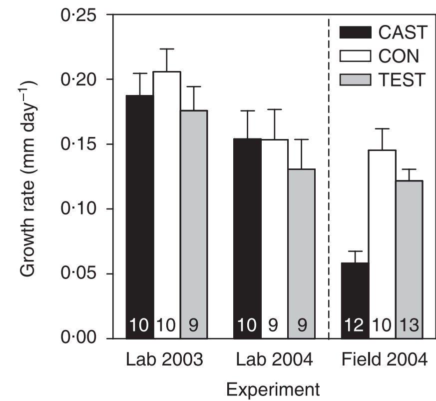

Fig. 4. Mean (± 1 SE) growth rate of male treatment groups in the 2003 and 2004 laboratory experiments. Field 2004 growth rates from Cox & John-Alder (2005) are presented for comparison. These field data were obtained at the same time of year (September–October) from animals of comparable age (3–4 months) as those used for the laboratory studies. Sample sizes are indicated at the base of each bar.

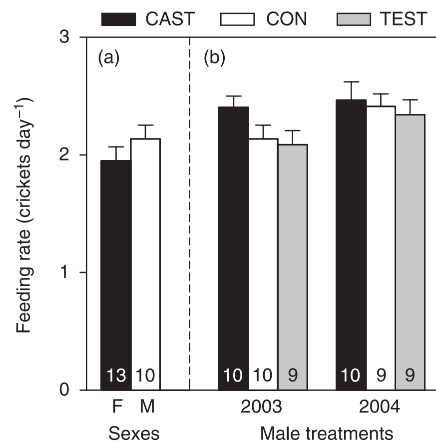

Fig. 5. Mean (± 1 SE) feeding rate by sex (a) and male treatment group (b). Food availability was three crickets day^{-1} (maximum *y*-axis value). Sample sizes are indicated at the base of each bar.

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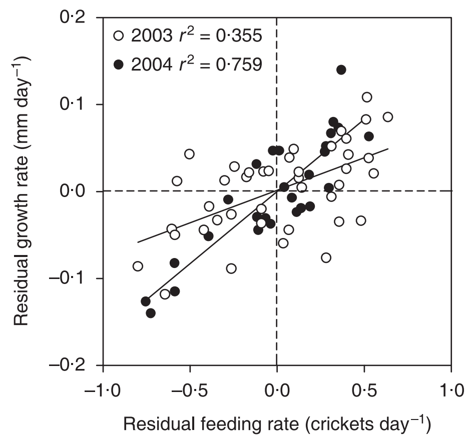

Fig. 6. Correlations between residuals obtained from least-squares regressions of feeding rate and growth rate on initial snout–vent length. Positive values indicate animals that ate (x-axis) or grew (y-axis) more than predicted for their body size. Residuals were obtained by pooling females and males of all treatment groups within each year.

EFFECTS OF FEEDING ON GROWTH

We calculated 'size-corrected' residual measures of individual growth rate and feeding rate by regressing each variable on SVL, with separate regressions for 2003 and 2004. Pooling sex and treatment groups, we found strong positive relationships between residual feeding rate and residual growth rate in both 2003 ($r^2 = 0{\cdot}355$; $P < 0{\cdot}001$; Fig. 6) and 2004 ($r^2 = 0{\cdot}759$; $P < 0{\cdot}001$; Fig. 6). Thus, for any given size, individuals that ate more crickets grew more quickly than those that ate fewer crickets. This result is not an artefact of pooling sex and treatment groups, since we found significant positive correlations ($P < 0{\cdot}05$) between residual feeding rate and residual growth within each individual sex and treatment group, with the exception of TEST males in 2003 ($P = 0{\cdot}68$).

Discussion

SEX DIFFERENCES IN GROWTH

Under natural environmental conditions, *Sceloporus jarrovii* males grow more quickly than females as yearlings, such that males average 10% larger than females as adults (Cox 2005; Ruby & Dunham 1984). In this study, we raised males and females in captivity in order to determine whether this natural sex difference in growth is expressed when both sexes experience identical environmental conditions. We did not observe a sex difference in growth of captive yearlings (Fig. 1; Table 1), and the development of SSD was consequently suppressed (Fig. 2). Our experiment was initiated after the onset of natural sexual growth divergence and conducted over a 37-week period, by the end of which captive animals had attained ages and sizes at which SSD is pronounced in the natural population. Thus, our experiment was of sufficient duration and appropriate ontogenetic timing to permit relevant comparisons with the natural development of SSD. Our experiment was initiated after the sexes had begun to diverge in size, so we do not know whether SSD can be completely eliminated by raising males and females in captivity from birth. Interestingly, Smith *et al.* (1994) found that neonatal *S. jarrovii* females actually grew more quickly than males in captivity, corroborating our finding that sex differences in growth are plastic with respect to laboratory *vs* field environments.

The suppression of SSD in captivity was mediated by the inhibition of male growth rather than the stimulation of female growth (Fig. 3). This differs from previous laboratory studies of other species in which the expression of SSD was suppressed via the stimulation of growth in the smaller sex (Woolbright 1989; Haenel & John-Alder 2002; Taylor & DeNardo 2005). Thus, unlike the situation in congeneric *S. undulatus* (Haenel & John-Alder 2002), we have no direct evidence that *S. jarrovii* females share the same physiological (i.e. genetic) growth potential as males. However, the high growth rate characteristic of free-living *S. jarrovii* males clearly depends on environmental context. Although *S. undulatus* and *S. jarrovii* differ with respect to the direction of SSD, it is interesting that in both species, captivity alters the growth rate of males but not females (see Haenel & John-Alder 2002).

One potential explanation for the suppression of SSD in captivity is that males were prevented from attaining natural growth rates due to energy limitation. However, this explanation is contradicted by the fact that no animal ever approached a feeding rate equivalent to food availability (Fig. 5). Furthermore, most captive animals appeared visibly obese throughout the study (R. M. Cox, personal observation). Thus, although growth in captivity was strongly tied to food consumption (Fig. 6), it seems unlikely that growth was limited by energy availability *per se*. Although captive *S. jarrovii* males and females did not differ in feeding rate (Fig. 5), their similar laboratory appetites may not be representative of natural sex differences in energy acquisition. In the field, yearling males consume more prey items than females (Simon 1976), and males have larger annual energy budgets than females of equal size (Congdon 1977). This suggests that natural sex differences in growth may reflect an underlying sex difference in energy acquisition. However, given the similarity in feeding rates of captive males and females, any natural sex difference in energy acquisition must be driven by environmental factors that were absent from our laboratory common garden.

In addition to increased energy acquisition, free-living males may support their higher growth rates by allocating a relatively larger fraction of available energy to growth. Given that captive males and females exhibited similar rates of energy intake for any given body size, their equivalent growth rates argue that fractional allocation to growth was similar between sexes. Our laboratory conditions probably eliminated most of the energetic costs normally associated with foraging, social behaviour, thermoregulation, reproduction and predator avoidance.

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Given that neither energy acquisition nor the potential for energy allocation trade-offs with activity differed between captive males and females, it is perhaps not surprising that we failed to observe a sex difference in growth.

TESTOSTERONE AND MALE GROWTH

Recently, we demonstrated that castration can stimulate male growth in *Sceloporus* species with female-larger SSD, whereas treatment with exogenous T inhibits male growth in these species (Cox & John-Alder 2005; Cox *et al*. 2005a). By contrast, we found that castration inhibits growth in free-living *S. jarrovii* males, while exogenous T restores growth of castrated males to the rate of intact controls (Cox & John-Alder 2005; also see Fig. 4). These findings raise the intriguing possibility that T may act as a bipotential growth regulator by stimulating growth in species with male-larger SSD and inhibiting growth in species with female-larger SSD. Testosterone is known to stimulate growth in numerous mammals, fish and birds (see References in Cox & John-Alder 2005). By contrast, nearly every study involving reptiles has found either no effect or an inhibitory effect of T on growth (reviewed in Cox & John-Alder 2005), regardless of underlying differences in SSD, reproductive mode or phylogenetic placement. Although some previous studies have acknowledged concerns about the relevance of growth inhibition in the light of possible pharmacological T levels (Hews *et al*. 1994; Lerner & Mason 2001), we have repeatedly found that our implants reliably elevate plasma T levels within the natural physiological range for *Sceloporus* males (Cox & John-Alder 2005; Cox *et al*. 2005a; see below). Further, we have observed both inhibition and stimulation of growth by T in closely related species using identical implants and surgical procedures. Thus, we believe that our unique results reflect real biological differences (rather than methodological artefacts) that raise interesting questions about sex-specific endocrine growth regulation in reptiles.

In the present study, we saw pronounced treatment effects on plasma T levels and secondary sexual characteristics that are known to respond to androgenic stimulation (Table 2). Castration consistently reduced plasma T to levels typical of females, while implants restored plasma T to levels (mean 39·04 ng ml^{-1}) that are well within the normal physiological range for free-living yearling males during the fall breeding season (mean 55·21 ng ml^{-1}; Cox & John-Alder 2005). Unexpectedly, we did not observe any effects of castration or exogenous T on growth rates of captive males in either of two separate experiments (Fig. 4). Why did growth responses to castration and exogenous T differ between the field and captivity? A comparison of our results from 2004 is informative in this regard, since growth rates of CON and TEST males were nearly identical in either environment. By contrast, growth rates of CAST males were about 2·6 times higher in captivity than in the field (Fig. 4). This suggests that treatment effects were absent in captivity because CAST males were able to attain growth rates similar to TEST and CON males, not because growth rates of CON and TEST males were suppressed.

The absence of growth inhibition by castration is unexpected given our previous results for free-living males, but not surprising when one considers that captive treatment groups probably did not differ in environmentally mediated energy acquisition or allocation trade-offs that might constrain growth. This explanation illustrates the environmental sensitivity of sex-specific endocrine growth regulation and directs interest squarely on the question of how energy acquisition and/or allocation decisions of free-living males differ among treatment groups. In particular, it would be informative to know how castration inhibits growth in the field, since it is clear from our laboratory results that high circulating T levels are not prerequisite for high levels of energy acquisition and allocation to growth. One possibility is that castration introduces energetic costs that are traded off against growth in the field but are absent or mitigated by energy surplus in the laboratory. However, the available data do not support this interpretation. Exogenous T stimulates activity and aggression in free-living *S. jarrovii* males, thereby increasing total metabolic expenditure (Marler & Moore 1988, 1989, 1991; Marler *et al*. 1995). Because castration inhibits the expression of aggressive behaviours (Moore 1987, 1988), it would probably minimize any associated metabolic costs that might constrain growth.

A second, non-exclusive possibility is that castration inhibits growth in free-living males by altering tissue-specific energy deposition. In mammals, castration typically shifts energy allocation toward fat deposition, whereas T increases lean muscle mass (reviewed in Ford & Klindt 1989). Females of *S. jarrovii* typically exceed males in allocation to lipid storage (Ballinger 1973), and castration may shift male allocation toward the female pattern. However, we observed an inhibition of mass gain in response to castration of free-living males (Cox & John-Alder 2005), suggesting that castration reduced not only growth in length, but also total allocation to tissue production. Further, we found no difference in wet mass of abdominal fat bodies among captive male treatment groups, suggesting that tissue-specific allocation to both storage and growth was similar among treatments.

Castration could also inhibit growth in free-living males by decreasing their energy acquisition. This seems counterintuitive, since intact, free-living *S. jarrovii* males treated with T reduce feeding in favour of increased aggression and courtship (Marler & Moore 1989, 1991; Klukowski *et al*. 2001). Thus, castration should facilitate increased feeding by removing the primary endocrine stimulus for these time-consuming social behaviours. However, it is also possible that castration decreases daily activity period (Cox *et al*. 2005a), and thus the amount of time available for foraging. During the autumn, *S. jarrovii* males are much more active than females, and their larger time budgets may allow for increased

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foraging to support their larger overall energy budgets (Congdon 1977). To the extent that T mediates this sex difference in activity, castration would be expected to produce a time and energy budget similar to that of females, with a resultant decrease in energy available for growth. A shorter daily activity period may also reduce the amount of time that females or castrated males are able to maintain high body temperatures necessary for maximal growth (see Niewiarowski & Roosenburg 1993). Even if such sex and treatment differences in daily activity period were to occur in captivity (see Klukowski *et al.* 2004), it is unlikely that they would affect feeding rate or body temperature in our simplified laboratory environment. Thus, if sex and treatment differences in growth of free-living animals reflect underlying differences in activity period, it is not surprising that we failed to observe growth differences in captivity.

Conclusions

In *Sceloporus* lizards, SSD develops because of sex differences in growth rate. Sexual growth divergence is temporally correlated with divergence in plasma T levels, and hormone manipulations in the natural environment have shown that male growth rate is strongly influenced by T (Cox & John-Alder 2005; Cox *et al.* 2005a). Species differences in growth responses to T may reflect fundamental differences in the effects of T on the endocrine growth axis, which is influenced by sex steroids in other vertebrate classes. Similar endocrine mechanisms may differentially regulate male and female growth to produce adaptive sex differences in body size. However, growth inhibition by T is probably not adaptive with respect to body size *per se*, since relatively larger males achieve greater reproductive success even in species where females are the larger sex (see Cox & John-Alder 2005). The general alternative is that T affects growth indirectly, by introducing context-dependent energy acquisition and/or allocation trade-offs. Results of the present study support this conclusion from two different angles: (1) natural sex differences in growth rate are absent in a laboratory common garden environment and (2) castration and exogenous testosterone have no effect on male growth in captivity, whereas castration inhibits male growth in the field. These results call attention to the environmental sensitivity of sex-specific endocrine growth regulation and illustrate the importance of combining field and laboratory studies of growth and SSD.

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