Effects of food restriction on growth, energy allocation, and sexual size dimorphism in Yarrow's Spiny Lizard, *Sceloporus jarrovii*

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Abstract: Evolutionary biologists often view sexual size dimorphism (SSD) as a fixed genetic consequence of sexually antagonistic selection, but the actual magnitude of SSD may often be strongly dependent upon proximate environmental factors. Sexual differences in growth rate lead to male-biased SSD in wild populations of Yarrow's Spiny Lizard (*Sceloporus jarrovii* Cope, 1875), yet both sexes grow at similar rates under controlled laboratory conditions. We hypothesized that male-biased SSD in *S. jarrovii* reflects an obligatory sexual difference in energy allocation to growth versus competing functions, but that an ad libitum diet provides an energy surplus which overwhelms this sex-specific energetic trade-off. To test this hypothesis, we reared juveniles under high (3 crickets/d) and low (1 cricket/d) food availabilities. Food restriction dramatically reduced growth in both sexes but did not differentially affect growth of females relative to males. Food consumption did not differ between sexes, but males grew slightly faster than females at both levels of food availability, indicating a greater fractional allocation of available energy to growth. By contrast, females had larger fat bodies than did males, particularly under food restriction. This sexual difference in energy allocation to storage could explain the slightly higher growth rate of males relative to females.

Résumé : Les biologistes qui étudient l'évolution considèrent souvent le dimorphisme sexuel de la taille (SSD) comme une conséquence génétique fixe d'une sélection différente en fonction des sexes; cependant, l'importance réelle du SSD peut souvent être fortement dépendante des facteurs environnementaux proximaux. Des différences sexuelles du taux de croissance chez les populations sauvages du lézard épineux de Yarrow (*Sceloporus jarrovii* Cope, 1875) entraînent un SSD qui favorise les mâles, bien que les individus des deux sexes croissent au même taux dans les conditions contrôlées de laboratoire. Nous formulons une hypothèse selon laquelle le SSD qui favorise les mâles chez *S. jarrovii* reflète une différence sexuelle obligatoire dans l'allocation des ressources à la croissance par rapport aux fonctions de compétition; par ailleurs, un régime alimentaire ad libitum crée un surplus d'énergie qui compense ce compromis énergétique spécifique au sexe. Afin de tester notre hypothèse, nous avons élevé des jeunes lézards dans des conditions de disponibilité de nourriture forte (3 grillons/j) ou faible (1 grillon/j). Une restriction de la nourriture réduit considérablement la croissance chez les deux sexes, mais n'affecte pas la croissance des femelles différemment de celle des mâles. La consommation de nourriture ne diffère pas entre les sexes, mais les mâles croissent un peu plus rapidement que les femelles aux deux niveaux de disponibilité de nourriture, ce qui indique l'allocation d'une fraction relativement plus importante de l'énergie disponible à la croissance. En revanche, les femelles possèdent des corps gras plus volumineux que ceux des mâles, particulièrement lorsqu'il y a restriction de nourriture. Cette différence sexuelle dans l'allocation de l'énergie aux réserves pourrait expliquer le taux de croissance légèrement plus élevé chez les mâles que chez les femelles.

[Traduit par la Rédaction]

Introduction

Sexual differences in body size (sexual size dimorphism; SSD) are nearly ubiquitous across most animal taxa, and biologists dating back to Darwin (1871) have sought to understand the selective pressures that drive the sexes apart (reviewed in Fairbairn et al. 2007). This evolutionary perspective is structured around the assumption that phenotypic SSD reflects underlying sexual differences in the genetic basis for body size. This is certainly true in many situations, but recent research has emphasized the importance of proximate environmental (i.e., nongenetic) factors in shaping the magnitude of SSD within populations (Watkins 1996; Duvall and Beaupre 1998; Haenel and John-Alder 2002; Le Galliard et al. 2006; John-Alder et al. 2007). Adult body size is the result of a complex ontogenetic growth process, and environmental factors that influence the growth of one or both sexes can therefore accentuate or constrain the de-

Received 1 September 2007. Accepted 8 January 2008. Published on the NRC Research Press Web site at cjz.nrc.ca on 14 March 2008.

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 doi:10.1139/Z08-002

velopment of SSD (Taylor and DeNardo 2005; Cox 2006; Cox et al. 2006; Le Galliard et al. 2006). This underscores the emerging perspective that the evolution of SSD is intimately tied to the underlying developmental processes that give rise to sexual differences in growth (Badyaev 2002).

Environmental sensitivity of SSD has frequently been demonstrated by contrasting growth patterns in the wild with those observed under controlled laboratory conditions. For example, female-larger SSD develops in wild Coquis (*Eleutherodactylus coqui* Thomas, 1966) because males stop growing upon maturation, but captive males continue to grow and attain large body sizes typical of females (Woolbright 1989). Conversely, male-larger SSD develops in wild Western Diamond-backed Rattlesnakes (*Crotalus atrox* Baird and Girard, 1853) because the growth of females slows after maturation (Beaupre et al. 1998), but the development of SSD is suppressed in captivity because sexual differences in growth rate fail to emerge (Taylor and DeNardo 2005; John-Alder et al. 2007). Studies of both male- and female-larger species of *Sceloporus* lizards reveal similar environmental sensitivity of sexual differences in growth (Haenel and John-Alder 2002; Cox et al. 2006; Cox and John-Alder 2007*a*; John-Alder et al. 2007). Collectively, these studies suggest that males and females often share common genetic potentials for growth, and that SSD is the result of complex interactions between proximate environmental factors and evolved genetic differences between the sexes.

Despite an emerging focus on the environmental sensitivity of SSD (Watkins 1996; Cox et al. 2006; Le Galliard et al. 2006; Roitberg 2007), the precise behavioral, demographic, and energetic mechanisms that give rise to this phenomenon remain uncertain. However, recent research suggests that sexual differences in energetic trade-offs (e.g., energy allocation to growth versus reproduction) may be of general importance in the development off SSD, particularly in ectotherms with indeterminate growth patterns (Schultz 1993; Sugg et al. 1995; Cox and John-Alder 2005; Cox et al. 2005*a*; Taylor and DeNardo 2005; Cox 2006; Cox and John-Alder 2007*a*; John-Alder and Cox 2007). If males and females differ in patterns of energy acquisition and (or) allocation to growth, then environmental variation in energy availability could alter the development of SSD. For example, male Yarrow's Spiny Lizards (*Sceloporus jarrovii* Cope, 1875) are, on average, about 10% larger than females, and this adult SSD develops in the wild because juvenile males grow more quickly than females (Cox 2006; Cox and John-Alder 2007*a*). However, when males and females are raised in captivity under ad libitum food availability, sexual differences in growth are absent and the development of SSD is suppressed (Cox et al. 2006; John-Alder and Cox 2007; John-Alder et al. 2007).

We hypothesize that male-biased SSD in *S. jarrovii* reflects an obligatory sexual difference in energy allocation to growth versus competing functions, but that an ad libitum diet provides an energy surplus which overwhelms this sex-specific energetic trade-off. In the present study, we test this hypothesis by raising captive males and females under both ad libitum and restricted diets. If SSD is suppressed in captivity because energy surplus overwhelms sex-specific energy allocation trade-offs, then we predict that sex differences in growth should be more pronounced when energy availability is limited. By raising captive juveniles in social isolation, we remove any potential differences in energy allocation that arise from sexual differences in behavior and activity (i.e., metabolic costs). To investigate sexual differences in energy allocation to tissue biosynthesis (i.e., production costs), we compare body composition of males and females raised under each diet. If sexual differences in energy allocation to storage and (or) reproduction underlie differences in growth, then we predict that sexual differences in the wet mass of fat bodies (storage) and gonads (reproduction) should be more pronounced under food restriction. Since previous studies have shown that the sex steroid testosterone stimulates growth in this species (Cox and John-Alder 2005; John-Alder and Cox 2007), we also measured plasma testosterone levels of each animal to examine its relationship with growth rate and its response to food restriction.

Materials and methods

Animal collection and care

We collected *S. jarrovii* yearlings near Buena Vista Peak in the Chiricahua Mountains, Coronado National Forest, Arizona, USA (31°54′–31°55′N, 109°16′W). We collected 21 males and 20 females in September 2004, at which time yearlings are 2–3 months old and natural sexual differences in growth rate are pronounced (Cox 2005, 2006). Animals were transported to Rutgers University and housed individually in plastic cages (36 cm × 42 cm × 46 cm) 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 65W BR-40SP) above each basking site. Cages were arranged under a bank of fluorescent bulbs (General Electric Chroma 50) for ultraviolet radiation. Fluorescent lights were set on timers to provide a daily 12 h light : 12 h dark photoperiod and spotlights were set to provide a 10 h basking period. We separated individual cages with opaque barriers to prevent social interactions. Animals were collected under permit from the Arizona Game and Fish Department (SP 553889) and housed at Rutgers under permit from the New Jersey Division of Fish and Wildlife (SH 25086) and approval of the Rutgers University Animal Care and Facilities Committee (Protocol No. 01-019).

Experimental design

After acclimating animals to captivity for 1 week, we measured each individual's snout–vent length (SVL) to the nearest 1 mm with a ruler and its body mass to the nearest 0.02 g with an electronic balance. We then assigned each animal to one of four size-matched treatment groups: high-food males (n = 11), high-food females (n = 8), low-food males (n = 10), and low-food females (n = 12). These four groups did not differ in initial SVL ($F_{[3,37]} = 0.82$, $P = 0.49$) or body mass ($F_{[3,37]} = 0.24$, $P = 0.87$) prior to food manipulation. For 10 weeks thereafter, we provided high-food groups with 3 crickets/d, while restricting low-food groups to 1 cricket/d. Each week, we counted all live and dead crickets that remained in each cage and estimated feeding

rate (no. of crickets/d) for each animal by assuming that all missing crickets had been consumed. We measured SVL and body mass at biweekly intervals and estimated growth rate in SVL (mm/d) and body mass (g/d) as the slope of the linear regression of body size (SVL or mass) on elapsed time (d) for each individual lizard (Cox et al. 2005*a*, 2006). We verified that growth was linear over the duration of our 70 d experimental period, as assumed by this method. At the conclusion of our experiment, we dissected each animal and measured the wet mass (to the nearest 0.1 mg) of its heart, liver, kidneys, gut, gonads, and abdominal fat bodies. In particular, we were interested in using the wet mass of the gonads and fat bodies as crude estimates of energy allocation to reproduction and storage, respectively.

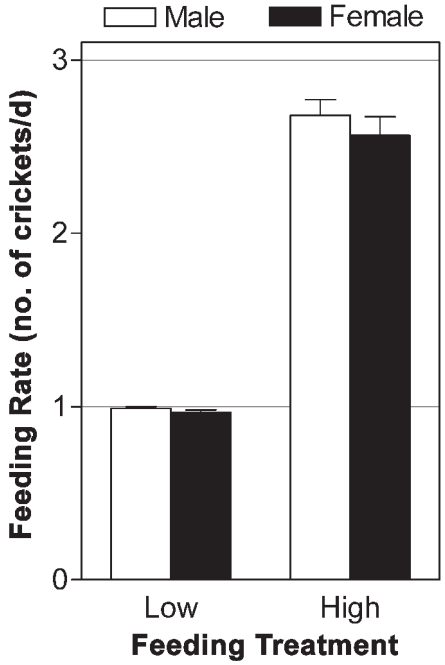
Testosterone assay

At the conclusion of our experiment, we used heparinized microhematocrit capillary tubes (Fisher Scientific, Pittsburgh, Pennsylvania) to collect blood samples from the postorbital sinus and from the trunks of animals as they were euthanized by decapitation for dissection. We held samples on ice until they could be centrifuged (within 2 h of collection) and then stored plasma at –20 °C until subsequent assays. We performed radioimmunoassays (RIAs) for plasma testosterone following methods reported elsewhere (Smith and John-Alder 1999; Cox and John-Alder 2005, 2007*b*; Cox et al. 2005*a*, 2005*b*). Samples were extracted twice in diethyl ether, dried under a stream of ultra-filtered air, and reconstituted in phosphate-buffered saline with gelatin (PBSG). Reconstituted samples were assayed with ^{3}H-testosterone as a radiolabel (PerkinElmer Life Sciences Inc., Boston, Massachusetts) and testosterone antiserum (1:18000 initial dilution) developed in rabbits by A.L. Johnson (The University of Notre Dame, Notre Dame, Indiana). We did not separate testosterone from other androgens prior to RIA, so our "plasma testosterone" values should be interpreted with the caveat that they reflect any additional binding of the testosterone antibody to 5α-dihydrotestosterone (DHT, 50% cross-reactivity). However, plasma DHT levels are typically only 2%–4% of plasma testosterone levels in this species (Woodley and Moore 1999), so our values primarily reflect testosterone. All samples were measured in a single assay. Interassay coefficients of variation are typically around 6% for this assay (Smith and John-Alder 1999). Our limit of detection was 2 pg of testosterone per assay tube.

Statistical analyses

We tested for sex and treatment effects using a two-way ANOVA with sex and treatment (high-food or low-food) as main effects, sex × treatment as an interaction term, and initial body size (SVL or mass) as a covariate. We analyzed the relationship between individual feeding rates and growth rates within each treatment group using an ANCOVA with size (SVL) as a covariate. To visualize the relationship between feeding rate and growth rate across individuals, we calculated size-independent residuals from the regression of $\log_{10}$(growth rate) or $\log_{10}$(feeding rate) on $\log_{10}$(initial body size) (i.e., SVL or mass), conducted separately for high- and low-food treatments. We analyzed body composition by $\log_{10}$-transforming the wet mass of the heart, liver, kidneys, gut, gonads, and fat bodies. We tested for effects of sex and treatment on organ masses using a two-way ANCOVA with sex and treatment as main effects, sex × treatment as an interaction term, and $\log_{10}$(final SVL) as a covariate. To determine the effects of sex and treatment on overall body composition, we used a MANOVA with sex and treatment as main effects, sex × treatment as an interaction term, and wet mass of each tissue as separate dependant variables. All statistical analyses were implemented in SAS® version 8.2 (SAS Institute Inc. 2001).

Fig. 1. Mean (+1 SE) feeding rate for male and female Yarrow's Spiny Lizards (*Sceloporus jarrovii*) in the high- and low-food treatment groups. Horizontal lines indicate food availability for high-food (3 crickets/d) and low-food (1 cricket/d) groups. Feeding rate differed by a factor of 2.7 between treatment groups but did not differ between sexes. Variance in the low-food group is slight because feeding rate equaled food availability for most animals.

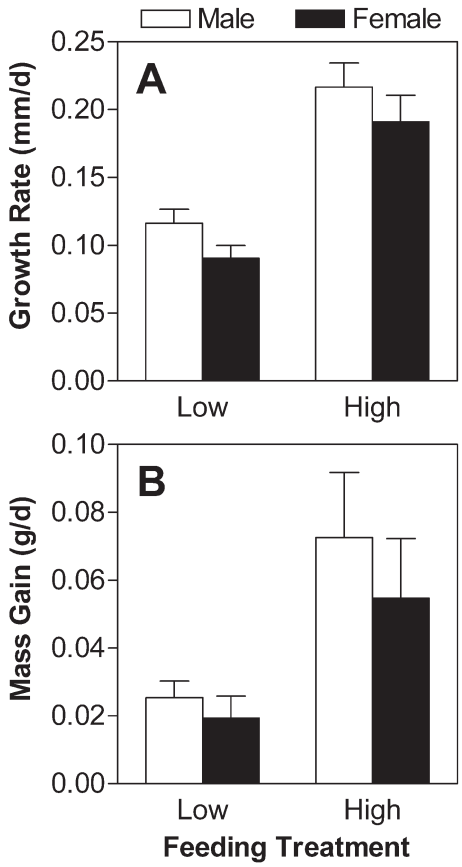


Results

Feeding rate

In the high-food treatment group, observed feeding rates were lower than food availability for both males and females (Fig. 1). Given that no individual ever achieved a consumption rate equal to food availability, we consider food availability to be effectively ad libitum for the high-food group. In the low-food group, observed feeding rates were nearly identical to food availability for both males and females (Fig. 1). Observed feeding rates were strongly dependent on treatment ($F_{[4,36]}$ = 707.76, P < 0.001) so that high-food animals consumed about 2.7 times as many crickets per day as low-food animals (Fig. 1). Feeding rate was independent of initial SVL ($F_{[3,36]}$ = 2.16, P = 0.15), but increased slightly as a function of initial body mass ($F_{[3,36]}$ = 4.98, P = 0.03), so we tested for effects of sex and the sex × treatment interaction using body mass as a covariate. However, we did not find any effect of sex ($F_{[4,36]}$ = 0.74, P = 0.39) or the sex × treatment interaction ($F_{[4,36]}$ = 0.33,

Fig. 2. Mean (+1 SE) rate of growth in (A) snout–vent length and (B) body mass for male and female Yarrow's Spiny Lizards (*Sceloporus jarrovii*) in each treatment group.

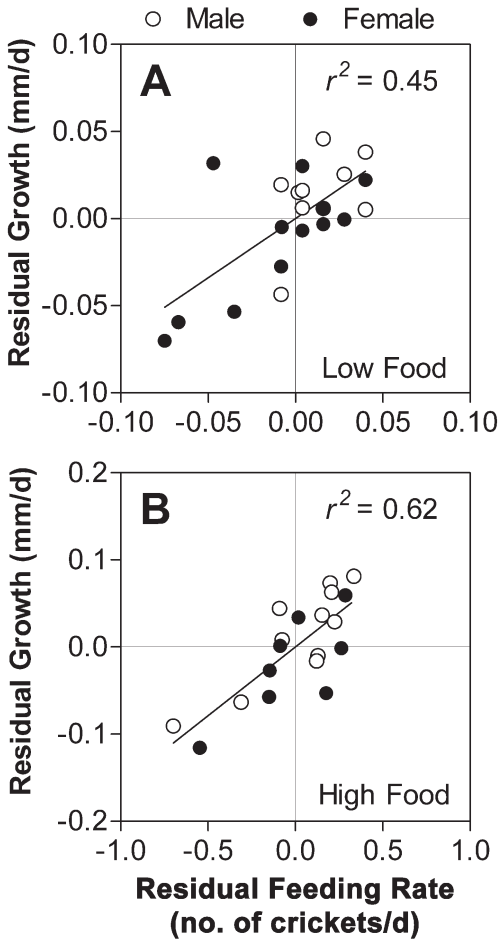


$P = 0.57$) on feeding rate, indicating that energy intake was similar in both males and females (Fig. 1).

Growth rate

Growth rate tended to decrease slightly as a function of initial SVL (mm/d; $F_{[4,36]} = 3.68$, $P = 0.06$) but was unrelated to initial body mass (g/d; $F_{[4,36]} = 0.18$, $P = 0.68$). Effects of sex, treatment, and the sex × treatment interaction were qualitatively similar regardless of whether or not we included initial SVL (when analyzing growth in millimetres) and body mass (when analyzing growth in grams) as covariates, so we report the results of our full a priori ANCOVA models. We observed strong treatment effects on growth rate in both SVL ($F_{[4,36]} = 52.67$, $P < 0.001$) and body mass ($F_{[4,36]} = 95.82$, $P < 0.001$). Overall, food restriction reduced growth rate by about twofold in SVL (Fig. 2A) and threefold in mass (Fig. 2B). For any given body size, animals with relatively high feeding rates also had relatively high growth rates, a result that was similar within both low-food (ANCOVA with SVL as covariate: $F_{[2,19]} = 15.79$, $P < 0.001$) and high-food ($F_{[2,16]} = 25.79$, $P < 0.001$) treatment groups (Figs. 3A, 3B). Contrary to our previous results for captive *S. jarrovii* (Cox et al. 2006), males grew significantly faster than females in body mass ($F_{[4,36]} = 7.65$, $P = 0.009$) and, to a lesser extent, in SVL ($F_{[4,36]} = 4.43$, $P = 0.04$). However, we did not observe a significant sex × treatment interaction for growth rate in SVL ($F_{[4,36]} = 0.14$, $P = 0.71$) or mass ($F_{[4,36]} = 1.86$, $P = 0.18$). Thus, food restriction inhibited growth rate to a similar extent in both sexes (Figs. 2A, 2B).

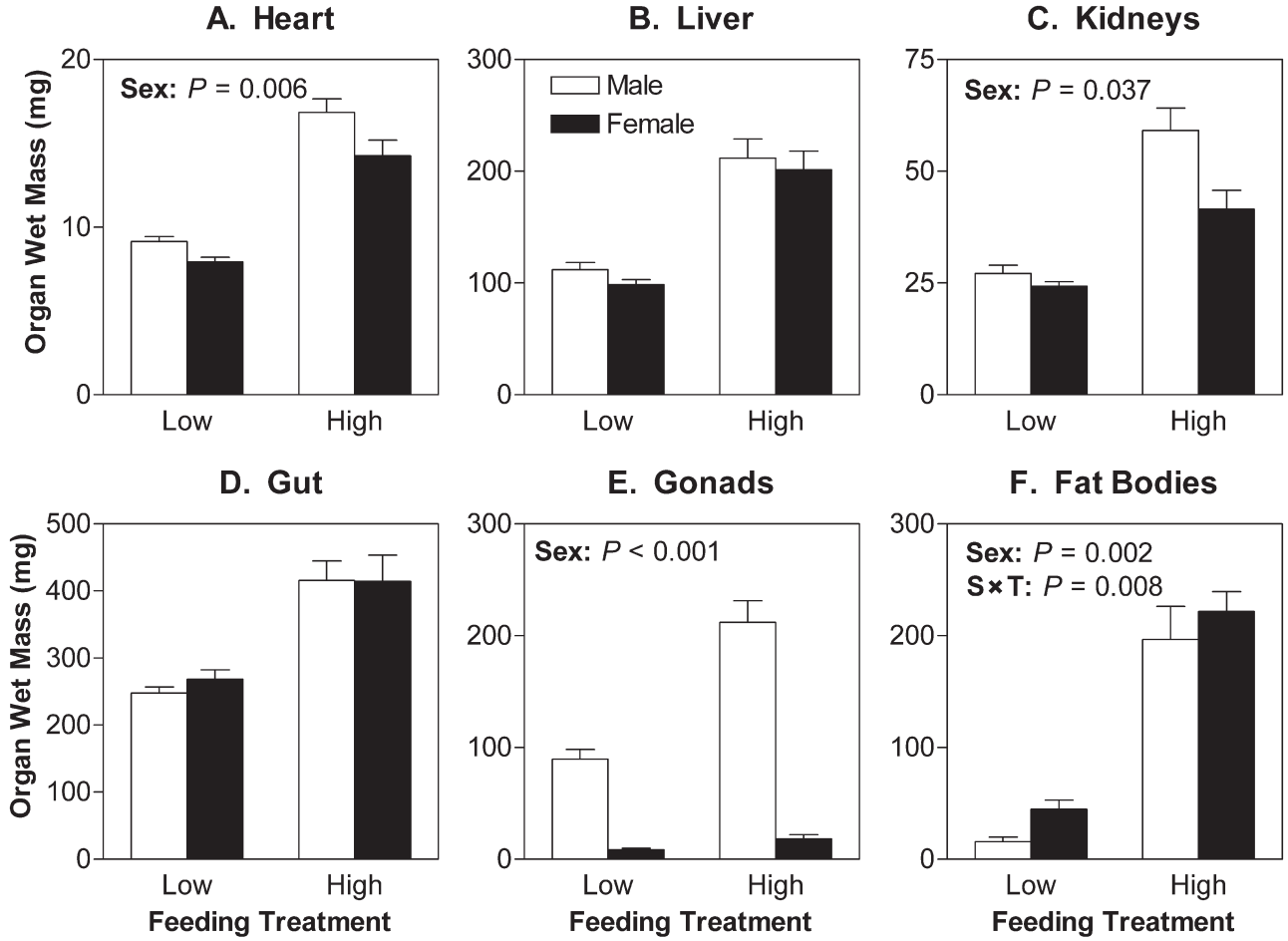
Fig. 3. Residuals from regressions of growth rate (*y* axes) and feeding rate (*x* axes) on initial snout–vent length for Yarrow's Spiny Lizards (*Sceloporus jarrovii*) separated by (A) low-food and (B) high-food treatments. Males and females were pooled within each treatment group to calculate residuals. Trend lines and statistics are reported for least-square regressions of residual growth rate as a function of residual feeding rate. For any given body size, growth rate increased with feeding rate in both treatment groups.



Body composition

Wet masses of heart, liver, kidneys, gut, gonads, and fat bodies increased with body size across all treatment groups ($P < 0.001$ for all comparisons). MANOVA showed that organ masses were strongly influenced by feeding rate ($F_{[6,31]} = 71.15$, $P < 0.001$) and sex ($F_{[6,31]} = 4.59$, $P = 0.002$), with no significant overall sex × treatment interaction ($F_{[6,31]} = 2.03$, $P = 0.091$). Separate ANCOVAs for each organ revealed strong effects of treatment on the mass of most organs ($P < 0.005$) except kidneys ($P = 0.04$) and gonads ($P = 0.91$). Thus, for a given body size, animals in the high-food group appeared to have somewhat larger hearts, livers, guts, and fat bodies than animals in the low-food group. However, these results should be interpreted with caution, since there was only slight overlap in body size between treatment groups.

Fig. 4. Mean (+1 SE) wet mass of (A) heart, (B) liver, (C) kidneys, (D) gut, (E) gonads, and (F) abdominal fat bodies for male and female Yarrow's Spiny Lizards (*Sceloporus jarrovii*) in the low- and high-food treatment groups at the conclusion of the experiment. Note the differences in scaling of the *y* axes among graphs. Statistics indicate significant effects of sex and the sex × treatment interaction (S × T) on organ mass, based on ANCOVA with $\log_{10}$-transformed data and $\log_{10}$(body size) (i.e., SVL) as a covariate.

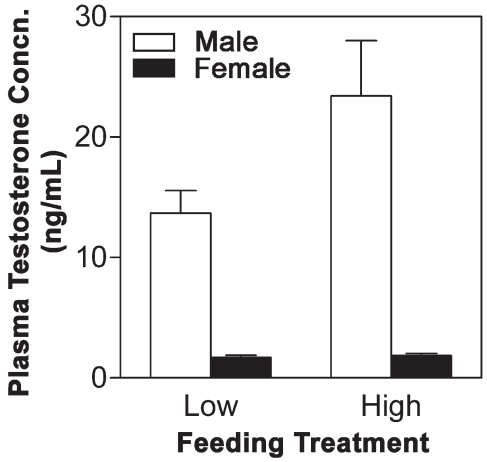


Sex effects were straightforward. For a given body size (SVL), males had larger hearts ($F_{[4,36]}$ = 8.76; P = 0.006), kidneys ($F_{[4,36]}$ = 4.72, P = 0.037), and gonads ($F_{[4,34]}$ = 317.71, P < 0.001) than females (Figs. 4A–4F). By contrast, the wet mass of abdominal fat bodies was greater in females than males ($F_{[4,36]}$ = 11.49, P < 0.002), and this sex difference was more pronounced under food restriction (sex × treatment interaction: $F_{[4,36]}$ = 3.47, P = 0.008; Fig. 4F). Fat bodies were the only organs to show a significant interaction between sex and treatment.

Plasma testosterone levels

Plasma testosterone levels were substantially greater in males than in females (ANOVA, sex: $F_{[3,35]}$ = 35.68, P < 0.001; Fig. 5). The effect of food restriction on circulating testosterone was weak and not significant (ANOVA, treatment: $F_{[3,35]}$ = 2.91, P = 0.10), and the sexual difference in plasma testosterone was not significantly affected by food restriction (ANOVA, sex × treatment: $F_{[3,35]}$ = 3.07, P = 0.09; Fig. 5). Plasma testosterone levels were uniformly low in females (range 1–3 ng/mL), so we restricted subsequent comparisons involving growth and testis mass to males. Based on their larger testes (Fig. 4E), we predicted that high-food males should have higher levels of circulating testosterone than low-food males. Despite substantial overlap in plasma testosterone levels between low-food (range 6–25 ng/mL) and high-food (range 4–46 ng/mL) males, our data indicate slightly higher mean testosterone levels in the

Fig. 5. Mean (+1 SE) plasma testosterone levels for male and female Yarrow's Spiny Lizards (*Sceloporus jarrovii*) in the low- and high-food treatment groups at the conclusion of the experiment.



high-food group (one-tailed t test: $t = 1.98$, $P = 0.035$). However, the range of plasma testosterone levels in low-food males indicates that food restriction did not simply cause "physiological castration" because of poor nutrition. Within the low-food treatment group, plasma testosterone levels were positively correlated with testis mass ($r^2 = 0.47$, $P = 0.029$), but we did not find a similar pattern in high-food males ($r^2 = 0.01$, $P = 0.92$). We did not find any correlation between plasma testosterone and growth rate (mm/d) in either male treatment group.

Discussion

Sexual differences in juvenile growth rates give rise to male-larger SSD in wild populations of *S. jarrovii* (Cox 2006; Cox and John-Alder 2007*a*; John-Alder et al. 2007). However, sexual differences in growth may be opposite or nonexistent in captivity (Smith et al. 1994; Cox et al. 2006). To explain this environmental sensitivity of growth and SSD, we hypothesized that sexual differences in growth reflect underlying differences in energy allocation to competing functions in the wild (i.e., growth, storage, maintenance, activity, reproduction), but that these sex-specific energetic trade-offs are overwhelmed by an energy surplus in captivity. Therefore, we predicted that experimental food restriction would reinstate sex-specific energy allocation trade-offs, leading to a sexual difference in growth at low, but not high, food availability.

Our results do not support this hypothesis. Although growth rate was strongly dependent upon food availability (Figs. 2, 3), food restriction did not differentially affect growth rates of males and females (Fig. 2). Similarly, other studies of squamate reptiles have shown that food restriction does not differentially impact the growth of males and females. In the Common Lizard (*Lacerta vivipara* Jacquin, 1787), a threefold reduction in food availability resulted in a threefold difference in SVL growth, but this treatment effect was similar in juveniles of both sexes (Le Galliard et al. 2005). In the Western Diamond-backed Rattlesnake, a similar dietary restriction also reduced growth dramatically, but failed to do so in sex-specific fashion (Taylor and DeNardo 2005).

Although food restriction did not differentially influence the growth of males and females, we did find evidence of sexual differences in energy allocation. Overall, males grew slightly faster than females at both levels of food availability, indicating that sexual differences in growth can persist even under ad libitum food availability. Previously, we reported the absence of sexual differences in growth under ad libitum food availability in captivity (Cox et al. 2006). The explanation for this discrepancy is unclear, since our previous study was conducted in the same captive facility and utilized juveniles of similar age from the same source population. In other reptiles, sexual differences in growth may persist under different levels of food availability (Le Galliard et al. 2005), or they may be entirely absent in captivity, regardless of food availability (Taylor et al. 2005). Our present results suggest the former scenario. Moreover, the fact that males grew slightly more quickly than females despite similar levels of energy acquisition in each sex indicates that males exceeded females in their fractional allocation of available energy to growth.

If sexual differences in energy allocation strategies influence SSD, then we should expect to find evidence for some competing aspect of the energy budget in which female allocation exceeds that of males. This expectation is met in the form of sexual differences in energy allocation to storage. Across both low- and high-food treatments, the wet mass of abdominal fat bodies was greater in females than in males (Fig. 4F), consistent with the observation that free-living *S. jarrovii* females exceed males in allocation to lipid storage (Ballinger 1973). Moreover, the sexual difference that we observed was accentuated under food restriction, such that low-food females had fat bodies that were three times larger than those of low-food males (Fig. 4F).

In *S. jarrovii*, fat bodies fluctuate in mass throughout the year, as energy is stored in adipose tissue during the summer monsoon season and expended on fall reproduction and winter maintenance (Congdon 1977). We did not measure fat bodies at the start of our experiment, so the sexual difference that we observed at its conclusion could reflect any combination of (*i*) differences that were present in the wild prior to our experiment, (*ii*) differences in fractional allocation to storage during captivity, or (*iii*) differences in use of stored energy to support other functions (e.g., growth) during captivity. For example, males and females in the high-food group may have been sated and increased their lipid stores during our experiment, whereas lizards in the low-food group may have drawn upon stored lipids to support other functions (e.g., growth, maintenance) in the face of energy limitation. We cannot distinguish among these various scenarios, but our data nonetheless demonstrate an overall sexual difference in the amount of energy stored as fat bodies (assuming equivalent energy densities of fat bodies in males and females; see Nagy 1983).

It is tempting to speculate that this sexual difference in the size of the fat bodies is functionally tied to sexual differences in growth, such that SSD develops at least in part as a consequence of underlying sexual differences in allocation to storage. However, males in the low-food group exceeded females, on average, by 419 mg in total mass gain, while females only exceeded males by 28 mg in wet mass of fat bodies. This discrepancy is even more pronounced in the high-food group, with a 1251 mg difference in total mass gain set against a 25 mg difference in wet mass of fat bodies. Even after accounting for the typically twofold greater energy density of fat bodies with respect to fat-free carcass (based on tissue dry mass; Nagy 1983), it seems unlikely that sexual differences in allocation to fat bodies per se can fully explain the observed difference in allocation to growth. However, abdominal fat bodies are only one potential site for lipid storage. To rigorously address this question, it would be necessary to know both initial and final energy contents of all somatic lipids and lean carcass tissues, as well as the respiratory energy required for biosynthesis of these tissues. In any case, our results remain informative as a general indication that allocation to storage differs between males and females of *S. jarrovii*.

Respiratory costs associated with reproduction (e.g., increased activity and territory defense in males) were essentially eliminated in our captive environment (Cox et al. 2006), but we found a striking sexual difference in the mass of reproductive tissues. At both high and low food availabil-

ities, the wet mass of the testes exceeded that of the ovaries by an order of magnitude (Fig. 4E), suggesting greater energy allocation to gonad development in males relative to females. This likely reflects sexual bimaturation characteristic of high-elevation populations of *S. jarrovii* (Ballinger 1979; Smith et al. 1994). Whereas males can attain physiological maturity (i.e., produce viable sperm) in the first autumn mating season (age 4–5 months), all females delay reproduction until their second mating season (age 16–17 months) at high elevations (Ballinger 1979; Smith et al. 1994). Although we do not know the actual energy content of testes and ovaries for quantitative comparison, this apparent difference in energy allocation to reproduction is in the opposite direction of the observed difference in allocation to storage. Therefore, sexual differences in allocation to gonad development cannot explain why males grew more quickly than females in our experiment. In fact, energy allocation to gonad development can severely constrain the growth of males, leading to female-larger SSD in species with sexual bimaturation (e.g., dwarf perch, *Micrometrus minimus* (Gibbons, 1854); Schultz 1993). This is clearly not the case in *S. jarrovii*, despite the presence of sexual bimaturation and a clear sexual difference in gonad size.

We have previously shown that testosterone stimulates growth in *S. jarrovii* (Cox and John-Alder 2005), but it does not appear that low growth rates in males can be attributed to low testosterone levels in the present study. Although plasma testosterone levels were lower in low- than in high-food males, both groups had testosterone levels well above those of females and castrated males in which growth is suppressed (Cox and John-Alder 2005). Moreover, sexual differences in growth are pronounced in free-living animals even when plasma testosterone levels of males are similar to those of our low-food group (Cox and John-Alder 2005). More to the point, we did not find any evidence that males with higher plasma testosterone levels also had higher growth rates in the present study. Finally, we have previously shown that effects of castration and testosterone replacement on growth of free-living males are not observed in captivity, suggesting that proximate environmental factors (i.e., food availability) overwhelm effects of testosterone on growth (Cox et al. 2006). Other studies have shown that sexual differences in growth can be eliminated in captivity, despite sexual differences in plasma testosterone and dramatic effects of food restriction on growth (Taylor and DeNardo 2005).

Overall, our results suggest that *S. jarrovii* males and females differ in energy allocation strategies but that energy surplus alone cannot explain why SSD does not develop in captivity (Cox et al. 2006). Our predictions were based on the rationale that ad libitum food availability in the laboratory greatly exceeds energy availability in the wild (see Smith et al. 1994), and that food restriction would more closely approximate energy availability in the wild. However, it is possible that our food-restriction treatment went too far in the opposite direction and severely constrained growth in both sexes, thus precluding the potential for sex-specific energy allocation decisions. Indeed, growth rates of wild males (0.21 ± 0.01 mm/d, mean ± 1 SE) and females (0.18 ± 0.01 mm/d; Cox 2006) of similar age are nearly identical to those that we observed in the high-food groups, but substantially higher than those of the low-food groups (Fig. 2). It is possible that, at some intermediate level of food availability, a sex-specific trade-off between growth and storage could result in a larger sex difference in growth rate than we observed in this study. However, our data suggest a very linear relationship between food consumption and growth in both sexes (Fig. 3). Given that growth rates of our high-food males and females were similar to those of free-living males and females, the most parsimonious interpretation is that our previous demonstration of equivalent growth rates in captive males and females was the result of a reduction in the growth of captive males, rather than an increase in the growth of females in response to increased energy availability (see Cox et al. 2006). However, this does not preclude the existence of sex-specific energy allocation trade-offs or their potential significance in the development of SSD (Cox et al. 2005*b*, 2006; Cox 2006; Cox and John-Alder 2007*a*).

Although our data from captive *S. jarrovii* indicate that males and females have similar feeding rates in captivity (Cox et al. 2006; Fig. 1), their similar laboratory appetites may not be representative of natural sexual differences in energy acquisition. In the wild, yearling males consume more prey items than females (Simon 1976), and males have larger energy budgets than females of equal size (Congdon 1977). This suggests that natural sexual differences in growth may reflect an underlying difference in energy acquisition. In light of present data, however, any natural sexual difference in energy acquisition must be driven by environmental factors absent from the laboratory. Moreover, while sexual differences in energy allocation may support the relatively higher growth rates of males in the wild, differences in energy intake are not prerequisite for sexual differences in growth: captive males grew slightly more quickly than females even when energy intake was equivalent in both sexes (Figs. 1, 2).

In summary, we have shown that growth rate is strongly dependent on energy intake in *S. jarrovii*, as in other lizards (Dunham 1978; Sinervo and Adolph 1989). However, food restriction does not differentially impact the growth of males and females, despite apparent sexual differences in energy allocation to growth versus competing functions (e.g., reproduction, storage). This result in of itself does not preclude the importance of sex-specific energy allocation trade-offs in the development of SSD (but see Cox 2006). However, it does suggest that the absence of sexually dimorphic growth in captivity (Cox et al. 2006) is not simply the result of energy surplus overwhelming inherent trade-offs that differentially constrain the growth of females. More likely, similar growth rates of males and females in captivity reflect more subtle environmental alterations in social and behavioral stimuli, thus precluding natural sexual differences in activity, foraging, and other factors that influence energy acquisition (Simon 1976; Klukowski et al. 2001). However, results from the present study contradict those of a previous captive study (Cox et al. 2006) by demonstrating that sexual differences in growth are present in captivity, even if somewhat muted with respect to differences observed in the wild (Ruby and Dunham 1984; Cox 2006; Cox and John-Alder 2007*a*). Our data suggest that sexual differences in energy allocation trade-offs between storage and growth underlie

the development of SSD in *S. jarrovii*, although a more rigorous quantification of energy allocation is necessary to fully address this issue. Collectively, our results contribute to an expanding literature on the proximate environmental factors that influence the development of SSD (Schultz 1993; Stamps 1995; Watkins 1996; Duvall and Beaupre 1998; Badyaev 2002; Rutherford 2004; John-Alder et al. 2007).

Acknowledgments

Jessica Malisch assisted with animal care. Portions of this study were conducted as part of a George H. Cook Senior Honors Thesis by M.M.B. Barry Jesse and Daphne Fairbairn provided helpful comments, and Peter Morin gave statistical advice. This project was funded by the American Museum of Natural History (Theodore Roosevelt Memorial Fund and SWRS Student Support Fund grants to R.M.C.), the Graduate School – New Brunswick at Rutgers University (Predissertation Travel Award to R.M.C.), the Society for Integrative and Comparative Biology (Grant-In-Aid of Research to R.M.C.), and the National Science Foundation (IBN 0135167 to H.J.-A.).

References

Badyaev, A.V. 2002. Growing apart: an ontogenetic perspective on the evolution of sexual size dimorphism. Trends Ecol. Evol. **17**: 369–378. doi:10.1016/S0169-5347(02)02569-7.

Ballinger, R.E. 1973. Comparative demography of two viviparous iguanid lizards (*Sceloporus jarrovi* and *Sceloporus poinsetti*). Ecology, **54**: 269–283. doi:10.2307/1934336.

Ballinger, R.E. 1979. Intraspecific variation in demography and life history of the lizard, *Sceloporus jarrovi*, along an altitudinal gradient in southeastern Arizona. Ecology, **60**: 901–909. doi:10.2307/1936858.

Beaupre, S.J., Duvall, D., and O'Leile, J. 1998. Ontogenetic variation in growth and sexual size dimorphism in a central Arizona population of the western diamondback rattlesnake (*Crotalus atrox*). Copeia, 1998: 40–47. doi:10.2307/1447700.

Congdon, J.D. 1977. Energetics of the montane lizard *Sceloporus jarrovi*: a measurement of reproductive effort. Ph.D. thesis, Arizona State University, Tempe.

Cox, R.M. 2005. Integrating proximate and ultimate causes of sexual size dimorphism in lizards. Ph.D. thesis, Rutgers University, New Brunswick, N.J.

Cox, R.M. 2006. A test of the reproductive cost hypothesis for sexual size dimorphism in Yarrow's Spiny Lizard, *Sceloporus jarrovii*. J. Anim. Ecol. **75**: 1361–1369. doi:10.1111/j.1365-2656.2006.01160.x. PMID:17032368.

Cox, R.M., and John-Alder, H.B. 2005. Testosterone has opposite effects on male growth in lizards (*Sceloporus* spp.) with opposite patterns of sexual size dimorphism. J. Exp. Biol. **208**: 4679–4687. doi:10.1242/jeb.01948. PMID:16326949.

Cox, R.M., and John-Alder, H.B. 2007*a*. Growing apart together: the development of contrasting sexual size dimorphisms in sympatric *Sceloporus* lizards. Herpetologica, **63**: 245–257. doi:10.1655/0018-0831(2007)63[245:GATTDO]2.0.CO;2.

Cox, R.M., and John-Alder, H.B. 2007*b*. Increased mite parasitism as a cost of testosterone in male striped plateau lizards, *Sceloporus virgatus*. Funct. Ecol. **21**: 327–334. doi:10.1111/j.1365-2435.2007.01251.x.

Cox, R.M., Skelly, S.L., and John-Alder, H.B. 2005*a*. Testosterone inhibits growth in juvenile male eastern fence lizards (*Sceloporus undulatus*): implications for energy allocation and sexual size dimorphism. Physiol. Biochem. Zool. **78**: 531–545. doi:10.1086/430226. PMID:15957108.

Cox, R.M., Skelly, S.L., Leo, A., and John-Alder, H.B. 2005*b*. Testosterone regulates sexually dimorphic coloration in the eastern fence lizard, *Sceloporus undulatus*. Copeia, 2005: 597–608. doi:10.1643/CP-04-313R.

Cox, R.M., Zilberman, V., and John-Alder, H.B. 2006. Environmental sensitivity of sexual size dimorphism: laboratory common garden removes effects of sex and castration on lizard growth. Funct. Ecol. **20**: 880–888. doi:10.1111/j.1365-2435.2006.01177.x.

Darwin, C. 1871. The descent of Man, and selection in relation to sex. J. Murray, London.

Dunham, A.E. 1978. Food availability as a proximate factor influencing individual growth rates in the iguanid lizard *Sceloporus merriami*. Ecology, **59**: 770–778. doi:10.2307/1938781.

Duvall, D., and Beaupre, S.J. 1998. Sexual strategy and size dimorphism in rattlesnakes: integrating proximate and ultimate causation. Am. Zool. **38**: 152–165.

Fairbairn, D.J., Blanckenhorn, W.U., and Szekely, T. (*Editors*). 2007. Sex, size and gender roles: evolutionary studies of sexual size dimorphism. Oxford University Press, Oxford.

Haenel, G.J., and John-Alder, H.B. 2002. Experimental and demographic analyses of growth rate and sexual size dimorphism in a lizard, *Sceloporus undulatus*. Oikos, **96**: 70–81. doi:10.1034/j.1600-0706.2002.10915.x.

John-Alder, H.B., and Cox, R.M. 2007. Development of sexual size dimorphism in lizards: testosterone as a bipotential growth regulator. *In* Sex, size and gender roles: evolutionary studies of sexual size dimorphism. *Edited by* D.J. Fairbairn, W.U. Blanckenhorn, and T. Szekely. Oxford University Press, Oxford. pp. 195–204.

John-Alder, H.B., Cox, R.M., and Taylor, E.N. 2007. Proximate developmental mediators of sexual size dimorphism: case studies from squamate reptiles. Integr. Comp. Biol. **47**: 258–271. doi:10.1093/icb/icm010.

Klukowski, M., Ireland, M., and Nelson, C.E. 2001. Interaction of testosterone and social stimuli on the feeding behavior of male mountain spiny lizards, *Sceloporus jarrovi*. Copeia, 2001: 966–971. doi:10.1643/0045-8511(2001)001[0966:IOTASS]2.0.CO;2.

Le Galliard, J.-F., Ferriere, R., and Clobert, J. 2005. Juvenile growth and survival under dietary restriction: are males and females equal? Oikos, **111**: 368–376. doi:10.1111/j.0030-1299.2005.14163.x.

Le Galliard, J.-F., Massot, M., Landys, M.M., Meylan, S., and Clobert, J. 2006. Ontogenic sources of variation in sexual size dimorphism in a viviparous lizard. J. Evol. Biol. **19**: 690–704. doi:10.1111/j.1420-9101.2006.01094.x. PMID:16674566.

Nagy, K.A. 1983. Ecological energetics. *In* Lizard ecology: studies of a model organism. *Edited by* R.B. Huey, E.R. Pianka and T.W. Schoener. Harvard University Press, Cambridge, Mass. pp. 24–54.

Roitberg, E. 2007. Geographic variation in sexual size dimorphism within a widespread lizard species. *In* Sex, size and gender roles: evolutionary studies of sexual size dimorphism. *Edited by* D.J. Fairbairn, W.U. Blanckenhorn, and T. Szekely. Oxford University Press, Oxford. pp. 143–153.

Ruby, D.E., and Dunham, A.E. 1984. A population analysis of the ovoviviparous lizard *Sceloporus jarrovi* in the Pinaleño mountains of southeastern Arizona. Herpetologica, **40**: 425–436.

Rutherford, P.L. 2004. Proximate mechanisms that contribute to female-biased sexual size dimorphism in an anguid lizard. Can. J. Zool. **82**: 817–822. doi:10.1139/z04-063.

SAS Institute Inc. 2001. SAS®. Version 8.2 [computer program]. SAS Institute Inc., Cary, N.C.

Schultz, E.T. 1993. Sexual size dimorphism at birth in *Micrometrus minimus* (Embiotocidae): a prenatal cost of reproduction. Copeia, 1993: 456–463. doi:10.2307/1447145.

Simon, C.A. 1976. Size selection of prey by the lizard, *Sceloporus jarrovii*. Am. Midl. Nat. **96**: 236–241. doi:10.2307/2424585.

Sinervo, B., and Adolph, S.C. 1989. Thermal sensitivity of growth rate in hatchling *Sceloporus* lizards: environmental, behavioral, and genetic aspects. Oecologia (Berl.), **78**: 411–419. doi:10.1007/BF00379118.

Smith, L.C., and John-Alder, H.B. 1999. Seasonal specificity of hormonal, behavioral, and coloration responses to within- and between-sex encounters in male lizards (*Sceloporus undulatus*). Horm. Behav. **36**: 39–52. doi:10.1006/hbeh.1999.1523. PMID:10433885.

Smith, G.R., Ballinger, R.E., and Nietfeldt, J.W. 1994. Elevational variation of growth rates in neonate *Sceloporus jarrovi*: an experimental evaluation. Funct. Ecol. **8**: 215–218. doi:10.2307/2389904.

Stamps, J.A. 1995. Using growth-based models to study behavioral factors affecting sexual size dimorphism. Herpetol. Monogr. **9**: 75–87. doi:10.2307/1466997.

Sugg, D.W., Fitzgerald, L.A., and Snell, H.L. 1995. Growth rate, timing of reproduction, and size dimorphism in the southwestern earless lizard (*Cophosaurus texanus scitulus*). Southwest. Nat. **40**: 193–202.

Taylor, E.N., and DeNardo, D.F. 2005. Sexual size dimorphism and growth plasticity: an experiment on the western diamond-backed rattlesnake (*Crotalus atrox*). J. Exp. Zool. **303A**: 598–607. doi:10.1002/jez.a.189.

Taylor, E.N., Malawy, M.A., Browning, D.M., Lemar, S.V., and DeNardo, D.F. 2005. Effects of food supplementation on the physiological ecology of female Western diamond-backed rattlesnakes (*Crotalus atrox*). Oecologia (Berl.), **144**: 206–213. doi:10.1007/s00442-005-0056-x.

Watkins, G.G. 1996. Proximate causes of sexual size dimorphism in the iguanian lizard *Microlophus occipitalis*. Ecology, **77**: 1473–1482. doi:10.2307/2265544.

Woodley, S.K., and Moore, M.C. 1999. Female territorial aggression and steroid hormones in mountain spiny lizards. Anim. Behav. **57**: 1083–1089. doi:10.1006/anbe.1998.1080. PMID:10328794.

Woolbright, L.L. 1989. Sexual dimorphism in *Eleutherodactylus coqui*: selection pressures and growth rates. Herpetologica, **45**: 68–74.