Sexual Size Dimorphism and Growth Plasticity in Snakes: an Experiment on the Western Diamond-Backed Rattlesnake (*Crotalus atrox*)

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ABSTRACT

We conducted an experiment to examine the effects of sex and food intake on growth, mass gain, and attainment of sexual maturity in Western Diamond-backed Rattlesnakes (*Crotalus atrox*). We also measured testosterone levels to determine whether testosterone might be involved in the male-biased sexual size dimorphism observed in this species. We collected neonate rattlesnakes and raised them in the laboratory for 2 years on either a high-intake diet (fed one mouse per week) or a low-intake diet (fed one mouse every 3 weeks). High-intake snakes grew and gained mass more rapidly than low-intake snakes, but males did not grow or gain mass more rapidly than females in either treatment group. High-intake snakes attained reproductive maturity earlier than low-intake snakes, indicating that size, not age, is the critical determinant of reproductive maturity. Males had higher levels of testosterone than females but did not grow more quickly, suggesting that testosterone may not affect growth in this species and may therefore not be the proximate determinant of sexual size dimorphism.

Darwin (1871) recognized that sexual size dimorphism (SSD) was prevalent among animal species and varied in both direction and magnitude. Mammals and birds tend most often to show male-biased SSD where males are larger than females (Andersson, '94). Reptiles are variable but typically show female-biased SSD where females are larger than males (Fitch, '81). Numerous ultimate explanations of SSD have been proposed, but Darwin's original hypotheses are still the most widely discussed: in species where females are larger, size may provide a fecundity advantage, whereas in species where males are larger, size may lend an intrasexual competition advantage (Darwin, 1871). A recent idea emerging from movements to integrate the fields of physiology, ecology, and evolutionary biology is that SSD and other life history characteristics cannot be fully understood without elucidation of the physiological mechanisms responsible for them (e.g., the "physiology/life-history nexus", Ricklefs and Wikelski, 2002). Specifically, to understand how selection operates upon a trait such as body size, we must understand how body size is determined on a proximate level in an organism's life (Duvall and Beaupre, '98).

In ectotherms, body size is strongly dependent on resource availability. Numerous studies have experimentally confirmed that snakes exhibit resource-dependent growth; that is, snakes that consume more food grow more quickly and/or gain mass more quickly than snakes that consume less food (Forsman and Lindell, '96; Scudder-Davis and Burghardt, '96; Bonnet et al., 2001). Reproduction may be similarly regulated by resource availability: snakes that consume more food may reproduce earlier or more often than snakes that face lower resource availability (Ford and Seigel, '94; Lourdais et al., 2002). The plasticity of growth in snakes may be an adaptation to variable environments, in which the ability to speed or slow growth in response to fluctuating resource availability may be favored by natural selection (Partridge and Harvey, '88; Forsman and Lindell, '96). However, the sexes may face different selection pressures that favor different growth patterns. For example, in most species of snakes, females are larger than males, presumably because large body

size often increases fecundity in females (Ford and Seigel, '89; Madsen and Shine, '94). It may therefore be favorable for females to grow faster and/or for longer time periods than males. Indeed, females grow faster than males fed the same diet in several taxa that show female-biased SSD, including *Boa* (D. Hardy Sr., personal communication) and *Nerodia* (Scudder-Davis and Burghardt, '96). However, to our knowledge, no one has examined whether males grow faster than females fed the same diet in species of snakes that show male-biased SSD, and few hypotheses exist regarding what physiological factors may be responsible for sexually dimorphic growth in these species. Beaupre (2002) hypothesized that the high energetic costs of reproduction in female rattlesnakes reduce the amount of energy that can be allocated to growth, resulting in smaller size relative to males. However, it is unknown whether this is the sole factor leading to SSD in rattlesnakes, or whether genetic limitations or other physiological factors such as sex hormones contribute to SSD.

The steroid hormone testosterone (T) is responsible for the large body size of males in many vertebrate taxa (reviewed in Bardin and Catterall, '81). It is an anabolic hormone with a variety of growth-promoting effects (Bardin and Catterall, '81; Staub and De Beer, '97). However, the growth-promoting effects of T are not consistent across taxa. In the mammalian literature, T promotes growth in those species in which males are the larger sex (e.g., the rat, Slob and Van Derr Werff Ten Bosch, '75) and inhibits growth in those species in which females are larger (e.g., the golden hamster, Swanson, '67). Like mammals, both male- and female-biased SSD are observed in snakes (Shine, '78; Andrews, '82). In garter snakes (genus *Thamnophis*), which exhibit female-biased SSD (Fitch, '81), T inhibits growth and thus leads to a smaller size of males than of females (Crews et al., '85; Lerner and Mason, 2001). In snakes with male-biased SSD, it is possible that T stimulates growth in males; however, this possibility has never been tested.

In this experiment, we examined the effects of diet and sex on growth in the Western Diamond-backed Rattlesnake (*Crotalus atrox*), a snake with male-biased SSD (Klauber, '72). *C. atrox* males and females appear to be born the same size and grow at the same rate in the field, but develop SSD at some later point, possibly after the time of sexual maturity (Beaupre et al., '98). Figure 1 is a schematic of such a growth trajectory, with a divergence in growth between males and females occurring after attainment of sexual maturity. Historically, scientists have speculated that males grow larger than females as a result of sexual selection (Shine, '78), since larger male pit vipers tend to win fights for females (e.g., Schuett, '97); however, the physiological mechanism responsible for the divergence in growth is unknown.

We raised juvenile *C. atrox* in the laboratory on high- and low-intake diets to test several hypotheses. First, we hypothesized that rattlesnakes exhibit resource-dependent growth, and that males and females differ in growth. We predicted that snakes consuming more food would grow faster than snakes consuming less food, and males would grow faster than females. Such a sex difference could result from several factors, including, but not limited to, differential energy assimilation, expenditure, or allocation. Second, we hypothesized that the timing of reproductive maturity is determined by resource availability, predicting that snakes consuming more food would attain maturity earlier than snakes consuming less food. Finally, we serially measured plasma T levels to determine whether the onset of intersexual differences in plasma T levels are concurrent with an onset in intersexual differences in growth. While not definitive, this result would aid in evaluating the possibility that T is a contributing factor to SSD in *C. atrox.*

MATERIALS AND METHODS

Animal collection and care

In August 2000, we collected 32 neonate *C. atrox* from a 10 km road in Pinal Co., AZ. These snakes had not yet fed, as meals are visually detectable in snakes of this age (<3 weeks). We also obtained 18 neonates from six pregnant female *C. atrox* collected from an area within 5 km of the road. We maintained the 50 neonates in a temperature-controlled chamber in individual cages (54 x 23 cm, Freedom Breeder, Turlock, CA) with subsurface heating at one end and water available ad libitum. When snakes reached ca. 60 cm snout-vent length (SVL), they were moved to similar but

larger (54 x 40 cm) cages. Snakes experienced the following heat/light schedules: mid-Apr to mid-Oct: ambient temperature = 25 ± 1°C, scotophase 700–1900, supplemental heat on constantly; mid-Oct to mid-Dec and mid-Feb to mid-Apr: ambient temperature = 25 ± 1°C, scotophase 700–1900, supplemental heat on during scotophase; mid-Dec to mid-Feb: ambient temperature = 16± 1°C, no scotophase, supplemental heat off (to simulate an overwintering period). Over the course of the 2-year experiment (August 2000–July 2002), six snakes either died or consistently refused food, so data from these animals were not included in the analysis. All animal use was approved by the Arizona State University Institutional Animal Care and Use Committee (Protocol 01-617R).

Experimental design

Snakes were randomly assigned to one of two treatment groups: high intake (14 females and 9 males) and low intake (9 females and 12 males). High-intake snakes received one mouse per week and low-intake snakes received one mouse every 3 weeks. Before feeding, we recorded the mass of each mouse. The size of mice was progressively increased to follow the growth of the snakes until about March 2001; thereafter, mice consistently weighed 25–35 g. All snakes within each treatment group consumed similar total masses of mice over the course of the study. Snakes were not fed during the overwintering periods (mid-Dec to mid-Feb).

In April 2002, coinciding with the natural mating season and when snakes were 1.5 years old, we conducted breeding trials to assess reproductive activity. We placed males in females' cages for 2-day periods, checked the pairs twice daily, and recorded any copulations. Snakes were

randomly rotated such that each male was paired with each female at least twice; however, high-intake males were paired only with high-intake females, and low-intake males were paired only with low-intake females. Following the breeding trials, we assessed female reproductive condition each month with ultrasonography, and measured the masses and snout-vent lengths of all resultant offspring.

Measurements and blood collection

Every 6 weeks, we collected a blood sample and measured the SVL (±0.1 cm) and mass (±0.5 g) of each snake. Blood collection always occurred between 0800 and 1200 to control for circadian rhythms in hormone levels. Snakes were weighed, coaxed into plastic tubes, and bled from the caudal vein (0.25–1.0 ml) with a heparinized syringe within 5 min of removal from their cages. Blood was immediately centrifuged, and plasma was collected and stored at -80°C until radioimmunoassay could be performed. We then placed the snakes in a squeeze box (Quinn and Jones, '74), traced a line on the plastic top following the spine from snout to vent, and measured the line with a cloth measuring tape (= SVL).

Radioimmunoassay

We assayed blood samples for T because it is the primary androgen in this species, and other androgens such as dihydrotestosterone (DHT) are found in direct proportion to T but in much smaller quantities (Schuett et al., 2002, in press). We measured plasma levels of T in half the blood samples from males (every other sampling period), and at select sampling periods for females (October 2000, January 2001, October 2001, January 2002). Plasma volumes of 20 µl were refrigerated overnight with distilled water and 2000 cpm of ^{3}H-testosterone (^{3}H-T, NEN Life Science Products Inc., Boston, MA, Catalogue NET553) for individual recovery determination. We utilized an etherethanol-hexane extraction protocol in order to remove proteins and lipids that might interfere with antibody–hormone interactions (Taylor et al., 2004). Samples were extracted in 3 ml diethyl ether, the ether fractions were removed and dried with a stream of nitrogen gas in a hot water bath, and the samples were resuspended in 1 ml 90% ethanol and refrigerated overnight. Samples were then extracted with 2 ml hexanes, the ethanol fractions were removed and dried with nitrogen gas, and the samples were resuspended in 0.5 ml assay buffer (phosphate-buffered saline with gelatin). We used 200 µl in duplicate for the assay and an additional 50 µl for individual recoveries. For the assay, we added 100 µl ^{3}H-T and 100µl antibody (Wein Laboratories Inc., Succasauna, NJ, Catalogue T-3003)

to each duplicate sample, 100% bounds, and triplicate standard curve, and refrigerated them overnight. This antibody exhibits moderate cross-reactivity with DHT; however, since DHT levels parallel T cycles and are so low in C. atrox in comparison to T (Schuett et al., in press), the significance of any cross-reactivity between T and DHT would be minor. We separated bound and unbound T with dextran-coated charcoal and added the bound fraction to scintillation vials. We added 3 ml scintillation fluid, waited 12 hr, and counted the samples in a Beckman scintillation counter. Final steroid levels were calculated from a cubic spline curve fitted to standard curve values, and sample values were adjusted for individual recoveries. Samples from males and females were analyzed in two separate assays; the intra-assay coefficient of variation was 10%, and the inter-assay coefficient of variation was 0.3%. Mean percent recovery was 60%, and accuracy was 98%. All samples had detectable levels of T.

Data analysis

Statistical tests were performed using SAS (SAS Institute, Cary, IN, version 8.2), and data were subjected to tests for normality, homogeneity of variances, and presence of outliers prior to inferential tests. SVLs and masses were lntransformed to homogenize variances, but the data in the figures are shown back-transformed to original values.

We analyzed growth (i.e., changes in SVL) using the GLM procedure in SAS to perform a repeated measures analysis of variance (RMANOVA) with sex and diet as between-subjects factors, time as the within-subjects factor, and ln-transformed SVL as the dependent variable. For mass gain, we performed a similar analysis, with ln-transformed mass as the dependent variable. Mauchly's Criterion for Sphericity was violated for both the SVL and mass analyses; we therefore used multi-variate Wilks' Lambda tests rather than univariate tests (O'Brian and Kaiser, '85). We performed Tukey post-hoc comparisons of SVL and mass between males and females in each treatment group at each time period.

We analyzed T data using the MIXED procedure to perform RMANOVA with sex and diet as between-subjects factors, time as the within-subjects factor, and T concentration (ng/ml) as the dependent variable. The fact that we did not measure female T levels at all of the time points that we measured male T levels (see radioimmunoassay section above) resulted in missing data for the females. The MIXED procedure allows analysis of data sets with missing data (Littell et al., '96). PROC MIXED inferences were made using the unstructured covariance structure because this minimized the Akaike's Information and Schwarz' Bayesian Criteria (Littell et al., '96). Post-hoc comparisons of T levels between males and females and between high- and low-intake males at each time period were made with univariate t-tests adjusted for an experimentwise Type 1 error rate of 0.05.

RESULTS

Growth and mass gain

At the beginning of the experiment, there were no significant differences in SVL between snakes in each treatment group, but they diverged by the second measurement period, with high-intake snakes growing faster in SVL than low-intake snakes (Fig. 2). The ANOVA model detected a significant effect of diet on SVL, but sex and the sex x diet interaction were not significant, indicating no overall sex differences in growth (Table 1). There were significant effects of time and the time x treatment interaction on snake SVL, indicating that SVL changed over time and that the high- and low-intake treatment groups changed differently over time. There was also a significant time x sex interaction, indicating that the sexes changed differently in SVL over time. These sex differences were manifest only in the low-intake group, where females were slightly shorter than males at the beginning of the experiment because small females were by chance assigned to the low-intake group during random assignment of subjects to groups. Tukey post-hoc tests showed that this sex difference was significant early in the experiment but disappeared over time (Fig. 2). The high-intake snakes showed no sex differences in SVL throughout the experiment.

Results for the mass gain analysis were similar. By the second measurement period, high-intake snakes were significantly heavier than low-intake snakes (Fig. 3). The ANOVA model detected a significant effect of diet on mass (Table 2). Sex did not significantly affect mass gain, but the sex-diet interaction was significant, indicating that the sexes gained mass differently in each treatment group. In the low-intake group, females weighed less than males at the beginning of the experiment but this difference disappeared over time, while in the high-intake group, females actually became heavier than males toward the end of the experiment (Fig. 3). There were significant effects of time and the time x treatment interaction on snake mass, indicating that mass changed over time and that the high- and low-intake treatment groups changed differently over time. However,
the time x sex interaction was not significant, which means that males and females gained mass similarly over time.

Testosterone levels

Males had higher plasma T levels than females, and high-intake males had higher T levels than low-intake males at several time periods (Fig. 4). There were significant main effects of time, treatment, and sex, and all interactions were also significant (Table 3). This indicates that T levels changed over time, were different between the treatment groups and between the sexes, and changed differently over time in each treatment group and sex. Post-hoc tests revealed that males and females had similar T levels before 1/01, and males had higher T thereafter (Fig. 4). Post-hoc tests also revealed that males in the two treatment groups had significantly different levels of T at three of eight measurement periods (January 2001, July 2001, January 2002). These differences appear to be the result of higher T peaks in snakes from the high-intake group during these months (Fig. 4). This pattern is similar to the two peaks in T in free-ranging males, except that the peaks in this experiment occurred in January and July, whereas they occur in March and August in the wild (Taylor et al., 2004). The difference in timing of the T peaks most likely reflects differences in environmental cues in the laboratory and field. By July 2001 (age = 1 year), the high-intake males had circulating levels of T similar to those of free-ranging adult males (Taylor et al., 2004). Females consistently had low levels of T, similar to free-ranging females (Taylor et al., 2004).

Sexual maturity

In the breeding trials, six high-intake females copulated with five high-intake males. In contrast, no low-intake snakes copulated. Of the six high-intake females that copulated, four became pregnant. Their litter sizes were similar to those of wild female C. atrox from a study site within 5 km of the area where snakes in this study were collected (lab: n = 4 litters; mean number of neonates = 4.0 ± 1.8; field: n = 18 litters; mean number of neonates = 4.5 ± 1.6; Taylor and DeNardo, in press). However, the offspring of the high-intake females were larger than offspring born to wild females (lab: n =15 neonates, mean SVL = 32.5 ± 1.2 cm, range = 30.8–34.5 cm; field: n = 81 neonates; mean SVL = 28.6 cm± 1.5, range = 19.5–35.0 cm; $t = -5.97$, $p < 0.0001$), and were also heavier than wild offspring (lab: mean mass = 30.8±5.9 g, range = 21.0–44.5 g; field: mean mass = 20.0 ± 3.6 g, range= 9.0–36.0g; $t=-7.68$, $p < 0.0001$).

DISCUSSION

In this study, we experimentally examined the growth trajectories of C. atrox, a species of snake with male-biased SSD. High-intake snakes grew and gained mass faster than low-intake snakes, but males did not grow or gain mass more quickly than females. These results support the hypothesis of resource-dependent growth but do not support the hypothesis of sex differences in growth associated with male-biased SSD. High-intake snakes reached reproductive maturity earlier than low-intake snakes, supporting the hypothesis that attainment of maturity is affected by resource availability rather than age. Finally, high-intake males had higher T levels than low-intake males, and all females had low T throughout the experiment. The presence of a sex difference in T

levels combined with a lack of a sex difference in growth suggests that T may not affect growth, and therefore may not be involved in SSD.

The high-intake snakes attained adult-typical SVLs in less than 1 year, whereas the low-intake snakes grew along a trajectory that more closely resembled that of wild C. atrox, which typically mature in 3–4 years (Fitch and Pisani, '93; Beaupre et al., '98). The smallest female and male we observed mating at our study site were 70 and 75 cm in SVL, respectively. The high-intake snakes in this experiment reached these sizes by 10 months of age and continued to grow much larger (Fig. 2), but males did not grow faster or become larger than females throughout the study. The high-intake females in this study averaged 100 cm SVL (range= 92.7–106.8 cm) at only 2 years of age, a size that greatly exceeds that of wild adult females at our study site (n = 67; mean SVL = 82.4 cm; range = 70–94 cm). In fact, the size of these high-intake females even exceeds that of adult males from the same site (n = 104; mean SVL = 95.3 cm; range = 75–131 cm; E. Taylor and D. DeNardo, unpublished data).

The results for mass were similar: high-intake snakes gained mass more quickly than low-intake snakes, but males did not gain mass faster than females. In fact, from October 2001 until the end of the study, the high-intake females actually weighed *more* than the high-intake males, a trend that conflicts with patterns observed in the wild. This mass increase was not the result of deposition of yolk into eggs in preparation for reproduction, as ultrasonography showed that reproductive females did not initiate vitellogenesis until April 2002. The mass difference may be the result of differential retention of water, differential assimilation of food, or some other unknown factor. At only 2 years of age, the mean mass of the high-intake females was 892g (range= 747–988g) far higher than that of wild females (n=67; mean mass = 360 g; range = 195–510 g) and even wild males (n=104; mean mass = 533 g; range = 220–1192 g; E. Taylor and D. DeNardo, unpublished data) at our study site. The lack of sex differences in growth and mass gain in snakes fed the same diet and housed under the same conditions suggests that the SSD observed in wild snakes is a plastic rather than fixed phenomenon.

Androgens (e.g., T) are involved in the expression of many sexually dimorphic characters, such as comb development in chickens (Rath et al., '96), dewlap and ventral patch coloration in tree lizards (Hews and Moore, '95), and sexual dimorphism of the vertebrate brain (Gorski et al., '78; Breedlove, '92). Testosterone generally contributes to the larger body size and muscle mass of males relative to females (Bardin and Catterall, '81; Joubert et al., '94). Since rattlesnakes exhibit male-biased SSD (Klauber, '72), it is possible that T may promote growth in the male C. atrox. If T is the physiological factor responsible for SSD, then increases in T associated with sexual maturity in males should lead to increased growth of males relative to females thereafter (Fig. 1). However, our findings are not consistent with this prediction in that, at maturity, males consistently had higher plasma T levels (typical of free-ranging adult males; Taylor et al., 2004) but never showed higher growth rates than females (Figs. 2–4). While we merely demonstrate a lack of correlation between intersexual differences in T levels and growth, these results suggest that T is not directly responsible for growth. It is possible that T indirectly affects SSD, for example by stimulating increased activity of males, which then could lead to increased foraging success and/or muscle mass, an effect which would not occur under laboratory conditions. However, activity also entails increased energy expenditure and therefore the dramatic difference in size between the male and female *C. atrox* is unlikely to be the result of differences in activity alone. Possible direct and indirect roles of T in growth must be further tested by experimentally manipulating T levels in both the laboratory-housed and free-ranging male *C. atrox.*

The fact that high-intake males had higher T levels than low-intake males likely reflects the fact that they attained reproductive maturity earlier. At the time of the breeding trials (April 2002), low-intake snakes were smaller in size than snakes we typically observe copulating in the wild,

whereas high-intake snakes were as large as the largest snakes we find in the wild. This supports the idea that there is a minimum size rather than a minimum age necessary for attainment of sexual maturity, an idea that has been supported in other studies on ectotherms. For example, Ford and Seigel ('94) found that female snakes of the genus *Elaphe* matured early when raised on a high-intake diet. The mechanism by which attainment of a certain body size permits sexual maturity is unknown. One possibility is that attainment of a certain SVL is correlated with accumulation of a critical amount of fat reserves. In mammals, the hormone leptin is secreted from adipose cells in proportion to the amount of fat present and signals the central nervous system to begin production of gonadotropins at the time of puberty (Yu et al., '97), which then initiate the cascade of reproductive events. Indeed, prepubertal female mice injected with leptin attain reproductive maturity at an earlier age and smaller size (Chehab et al., '97). The effects of leptin on reproduction or attainment of maturity in reptiles are unknown.

There are several problems with utilizing copulation and pregnancy as indices for sexual maturation. First, reproductive behavior is seasonal in *C. atrox*, and therefore snakes may have been physiologically capable of reproduction much sooner than we detected if the appropriate environmental stimulus (warming after a cool overwintering period) had been provided earlier. Thus, reproductive activity during this experiment confirms sexual maturity but does not necessarily reflect the onset of sexual maturity, which might be better estimated by size relative to known reproducing animals in the wild. It is possible that the low-intake snakes were capable
of producing viable gametes but did not copulate for another, unknown reason or that one sex in the low-intake group was mature but the other was not. However, combining our data for reproduction, male testosterone levels, and growth relative to free-ranging wild snakes provides convincing evidence that snakes that consume more food reach sexual maturity earlier. Further tests are necessary to more fully understand the onset of sexual maturity and the mechanisms that regulate it.

Our results show that *C. atrox*, like many other species of snake, exhibit resource-dependent growth. In turn, attainment of reproductive maturity depends on this resource-dependent growth. The high-intake snakes surpassed the average SVL and mass of wild snakes and became reproductively mature during the study, yet SSD never developed. In fact, our results show that female *C. atrox* are capable of attaining male-like body sizes under laboratory conditions, indicating that the SSD present in wild *C. atrox* (outlined in Fig. 1) may be the result of intersexual differences in energy intake and/or expenditure. The most likely factor that could contribute to the small size of females in the wild is the high cost of reproduction relative to males (Beaupre, 2002). Beaupre and Duvall ('98) showed that the reproductive female *C. atrox* have higher energy requirements than non-reproductive females (an average non-reproductive female snake requires 4593 J/ day, while a reproductive snake requires 6580 J/ day). Following parturition, females are emaciated and allocate acquired energy toward replenishing that lost during reproduction, and little growth occurs (Beaupre, 2002), resulting in slowed growth relative to males after maturity (Fig. 1). Thus, SSD in rattlesnakes may be the consequence of differential energy expenditure between the sexes, and may not be the result of sexual selection favoring growth in males. In our study, reproductive investment by some of the high-intake females did not lead to SSD, probably reflecting the extremely high energy intake of these animals relative to the resource-limited condition in nature. Further research regarding the mechanisms that control the allocation of energy into storage versus growth and the roles that such mechanisms play in SSD is needed. In addition, future research efforts should explore other possible factors that might contribute to the small size of females relative to males in natural populations, such as potential differences in energy acquisition, thermoregulation, or activity levels.

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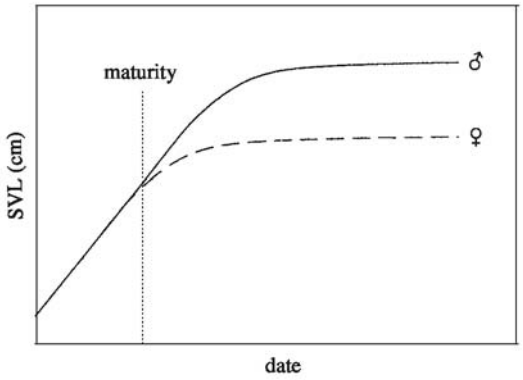

Fig. 1. Schematic of the development of sexual size dimorphism in Crotalus atrox. Juvenile snakes grow at the same rate until sexual maturity, after which males (solid line) grow faster than females (dashed line). The divergence in growth is caused by an unknown factor.

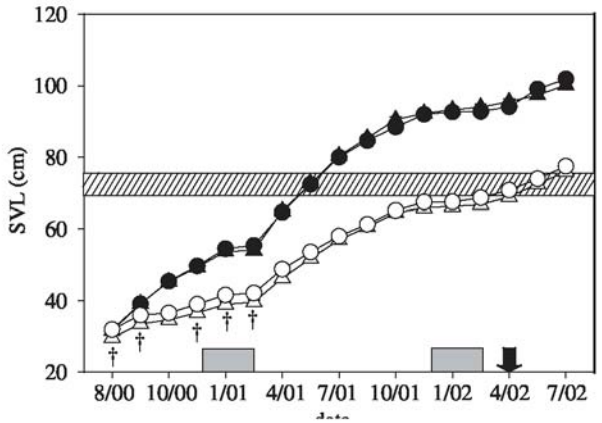

Fig. 2. Growth in snout-vent length (SVL) of Crotalus atrox. High-intake males are black circles and solid lines; high-intake females are black triangles and solid lines; low-intake males are white circles and dashed lines; low-intake females are white triangles and dashed lines. Significant sex differences in the low-intake group are marked by t. The shadowed bars correspond to overwintering periods during which snakes were not fed, the hatched bar denotes the approximate size at which free-ranging snakes attain sexual maturity, and the black arrow denotes the timing of the breeding trials. Values are shown as mean+1 SEM, although error bars are often invisible due to lack of variability among experimental units.

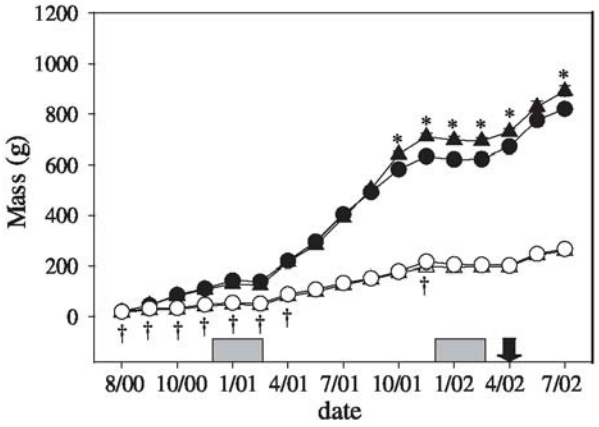

Fig. 3. Change in mass of Crotalus atrox. High-intake males are black circles and solid lines; high-intake females are black triangles and solid lines; low-intake males are white circles and dashed lines; low-intake females are white triangles and dashed lines. Significant sex differences in the low-intake group are marked by t, in the high-intake group by *. The shadowed bars correspond to overwintering periods during which snakes were not fed, and the black arrow denotes the timing of the breeding trials. Values are shown as mean+1 SEM, although error bars are often invisible due to lack of variability among experimental units.

TABLE 1. Repeated-measures ANOVA table for effects of diet, sex, time, and their interactions on ln-transformed SVL (cm) in Crotalus atrox

Between subjects	df	Type III S.S.	*F*-ratio	*P*-value
Diet	1	13.900	577.41	<0.0001
Sex	1	0.069	2.86	0.100
Diet × sex	1	0.062	2.59	0.116
Error	40	0.963		
Within subjects	**df**	**Wilks' λ**	***F*-ratio**	***P*-value**
Time	16.25	0.002	773.2	<0.0001
Time × diet	16.25	0.061	23.90	<0.0001
Time × sex	16.25	0.366	2.70	0.013
Time × sex × diet	16.25	0.474	1.73	0.106

TABLE 2. Repeated-measures ANOVA table for effects of diet, sex, time, and their interactions on ln-transformed mass (g) in Crotalus atrox

Between subjects	df	Type III S.S.	*F*-ratio	*P*-value
Diet	1	180.247	2627.17	<0.0001
Sex	1	0.264	3.84	0.060
Diet × sex	1	0.411	6.00	0.019
Error	40	2.744		
Within subjects	**df**	**Wilks' λ**	***F*-ratio**	***P*-value**
Time	16.25	0.002	965.42	<0.0001
Time × diet	16.25	0.026	59.02	<0.0001
Time × sex	16.25	0.538	1.34	0.248
Time × sex × diet	16.25	0.404	2.31	0.030

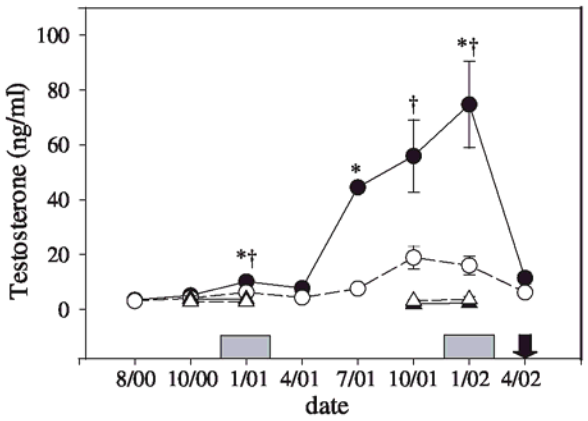

Fig. 4. Testosterone levels of male and female snakes in each treatment group. High-intake males are black circles and solid lines; high-intake females are black triangles and solid lines; low-intake males are white circles and dashed lines; low-intake females are white triangles and dashed lines. Significant differences between males and females are marked by t; those between high- and low-intake males by *. The shadowed bars correspond to overwintering periods, and the black arrow denotes the timing of the breeding trials. Values are shown as mean +1 SEM. Plasma T was only measured at four time periods in samples from females.

TABLE 3. Repeated-measures ANOVA table for effects of diet, sex, time, and their interactions on plasma testosterone levels (ng/ml) in Crotalus atrox

Between subjects	df	*F*-ratio	*P*-value
Diet	1.25	5.15	0.032
Sex	1.25	18.86	<0.001
Diet × sex	1.25	6.91	0.014
Within subjects	**df**	***F*-ratio**	***P*-value**
Time	7.25	75.93	<0.0001
Time × diet	7.25	46.28	<0.0001
Time × sex	3.25	6.75	0.002
Time × sex × diet	3.25	3.36	0.035