

AN ABSTRACT OF THE THESIS OF

Darren T. Lerner for the degree of Master of Science in Zoology presented on July 15, 1998. Title: The Ontogeny and Mediation of Sexual Size Dimorphism in the Red-spotted Garter Snake, *Thamnophis sirtalis concinnus*.

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Abstract approved: —

Robert T. Mason

A wide range of environmental and physiological factors influence the type and extent of sexual dimorphism found in animals. Influential factors include variable climate, competition for resources and mates, mate choice, and parental investment. In addition, some investigators attribute differences in body size to physiological factors influenced by genetic and environmental variation. Since Darwin's time researchers studying the evolution of animal life-history have discussed the interactions that exist between this wide array of influences on sexual dimorphism in the context of natural selection.

Much attention has been paid to the interrelationships of parental size, offspring size and number of offspring per reproductive bout. Spatial and temporal variation among these parameters have been investigated. However, these relationships have not been adequately examined while accounting for differences in offspring gender. In order to characterize the nature and extent of sexual size dimorphism found in adult red-spotted garter snakes, 108 adult females producing 782 female offspring and 790 male offspring

were utilized to examine the relationships of the number and size of offspring, clutch mass and maternal size.

The second facet of our investigation involved the hormonal manipulation of growth over the first year of post-natal life. To gain an understanding of which endogenous hormones may influence growth in this species we constructed a hormone profile of estradiol and testosterone from birth to 15 weeks of age. We experimentally tested the effects of exogenous hormone as well as the removal of endogenous hormone on growth with the use of estrogen and androgen antagonists.

We have found that the extent of sexual size dimorphism observed in adult red-spotted garter snakes is not present at birth. The relative number of males or females born varies differentially with maternal size and age such that larger, older females produce predominately more male offspring. There are no differences in endogenous levels of testosterone and estradiol from birth through 15 weeks and at 36 weeks of age between the sexes. However, these sex steroids do differentially affect the growth of males and females. Finally, we suggest that while all of these factors contribute to sexual size dimorphism in this species, embryonic and environmental influences need to be explored.

The Ontogeny and Mediation of Sexual Size Dimorphism in the Red-spotted Garter
Snake, *Thamnophis sirtalis concinnus*

by

Darren T. Lerner

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Darren T. Lerner, Author

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DEDICATION

This thesis is dedicated to my best friend and confidante Jennifer

The Ontogeny and Mediation of Sexual Size Dimorphism in the Red-spotted Garter Snake, *Thamnophis sirtalis concinnus*

CHAPTER I: GENERAL INTRODUCTION

The Evolution of Sexual Dimorphism

Theories regarding the evolution of differential body size stem from the assumption that size differences must play a role in driving and shaping the ecological and selective pressures on the biology and evolution of a species. While some authors evoke natural selection, others claim sexual selection explains the evolutionary pressures that influence sexual size dimorphism in animals. Sexual selection driven by such factors as parental investment and mate choice have been postulated to be the principal causes of sexual dimorphism (Darwin, 1871; Trivers, 1972; Brown, 1975; Wilson, 1975; Trivers, 1976). Darwin (1871) concluded that sexual selection arises from differences in reproductive success caused by competition for mates. Darwin suggested that females are larger than males in some animals, particularly in species that grow throughout life, because egg production increases with body size. He also indicated that when males are larger than females, as is common in many mammals, it is because large size is an advantage in contests for mates. There are many quantitative analyses that support these ideas (Selander, 1972; Ralls 1976; Shine, 1989).

Sexual selection cannot be implicated in shaping sexual dimorphism of morphological characteristics unless independent data show that they influence differential reproductive success (Cooper and Vitt, 1989). Others suggest that size

differences between the sexes may be a product of natural selection for ecological segregation of the sexes (Berry and Shine, 1980; Shine, 1989). Theoretical and empirical evidence indicate differential adaptations of foraging biology in the absence of competition between males and females (Slatkin, 1984; Shine, 1989). However, competition for resources may result in size dimorphism between the sexes, similar to that found between sympatric species (Hutchinson and MacArthur, 1959).

While some authors consider sexual selection to be a subset of natural selection, others make a clear distinction between the two. Darwin discussed this distinction between sexual selection and natural selection. "Sexual selection depends, not on a struggle for existence, but on a struggle between the males for possession of the females; the result is not death to the unsuccessful competitor, but few or no offspring" (Darwin, 1859, p.88).

In addition to these ultimate causes, proximate factors such as environmental temperatures, variation in food availability (Shine, 1987; Forsman, 1991) or mortality (King, 1989a) may play a role in shaping sexually dimorphic characteristics such as body size. Therefore, differences in body size can affect life history performances as well as relationships with environmental factors.

Sexual dimorphism associated with differential body size in adults is common in many animals. Among many species of birds and mammals, males are larger than females although this is not the rule (Selander, 1972; Ralls, 1977; Mueller, 1990). In avian species, exceptions are most prominent in the *Falconiformes* and *Strigiformes* in which adult females are the larger sex (Amadon, 1959; Olsen and Cockburn, 1991). Also not without exception, invertebrates and fishes tend to exhibit sexual size dimorphism in

favor of females (Darwin, 1871) as do many amphibians (Shine, 1979). For reptiles, females tend to be the larger sex in turtles and snakes, whereas in lizards and crocodylians males are more often the larger sex (Fitch, 1981).

Sexual Dimorphism in Snakes

Researchers have noted that dominance of one sex over the other with regard to adult body size is species-specific (Fitch, 1981; Ralls, 1976; Ralls, 1977; Shine, 1979). Similarly, the sex that attains larger adult size in snakes varies. In an extensive review of size dimorphism in snakes, Shine (1978) found that females were larger than males in two thirds of the species investigated. Males tend to be larger in species that exhibit male combat whereas larger female size is correlated with viviparity, large clutch sizes and residence within temperate climates (Fitch, 1981; Madsen and Shine, 1993a). However, male combat does not always lead to larger male size (Madsen, 1988; Forsman, 1991; Madsen and Shine, 1993b).

Insight into the evolution of sexual dimorphism in snakes not only involves investigations of the selective pressures behind sex differences in size, but also includes variation in body or head shape, coloration, scalation and ecology. Shine (1991) found head length (relative to body length) to be a sexually dimorphic character in nearly half of 114 snake species examined. In the red-sided garter snake, *Thamnophis sirtalis parietalis*, sexual dimorphism in relative head size has been attributed to gonadal hormones (Shine and Crews, 1988). These differences may be adaptive responses to selective pressures resulting in dietary differences between the sexes (Mushinsky, 1987;

Shine, 1993). In many snake species, males typically have longer tails than females after accounting for differences in body length (Klauber, 1943; Clark, 1967; King, 1989b). King reviewed data from 56 colubrid genera and found a correlation between species that favor larger females and longer tailed males. These findings support two major hypotheses regarding the evolution of these traits. The longer tail of males may be selected to provide larger housing for hemipenes and retractor muscles, while the shorter tails of females allows for a larger reproductive cavity by placing the cloaca in a more posterior position (Shine, 1993).

In general, differences in scalation are largely due to differences in shape or size. Variation in the position of the cloaca can contribute to differences in the number of subcaudal and ventral scales between males and females (Shine, 1993). Males of some species have tubercles or knobs under their chins. Gillingham (1987) suggested that these knobs might have a sensory function. Common to many snakes, males adpress their chins to the female's dorsum during courtship. Similar keeled scales are found in the area surrounding the cloaca among several taxa. These scales are also thought to perform a sensory or stimulatory function (Shine, 1993).

Unlike the extreme dichromatism seen in many birds and some lizards, snakes exhibit relatively subtle differences in color and pattern. Some of the more obvious dimorphic characters include differences in striping, banding or stippling. However, these differences are not predominately male or female traits. Males may be more brightly colored than females as juveniles, but just the opposite at maturity (Shine, 1993).

As there are no distinct trends within taxa, the ecological significance of color variation between the sexes (other than instances related to mimicry and crypsis of which there are a few) remains relatively unknown.

Little is known of the proximate causes of sexual dimorphism in snakes. In species with larger females, mechanisms for sexually dimorphic growth include delayed maturation in females, reduced growth rates after maturation in both sexes, and faster neonatal growth in females of some taxa (Andrews, 1982; Shine 1993). Neonatal growth is often similar between males and females, but significant sexual differences in juvenile growth rates have been reported in several species (Dmi'el, 1967; Platt, 1969; Crews, *et al.*, 1985; Madsen and Shine, 1993a). These differences in growth rates may be due to differences in feeding rates or metabolic expenditure. It is apparent that sexual size dimorphism is the result of a variety of mechanisms at variable stages of the life history among diverse taxonomic groups. More work is needed so that we may better understand the function and ecological impact of these mechanisms and how selective pressures influence their development.

Natural History of the Red-spotted Garter Snake

The common garter snake, *Thamnophis sirtalis*, exhibits adult sexual size dimorphism such that females are the larger sex. It is the most wide-ranging reptile in North America extending from Canada to Mexico and from the Atlantic to the Pacific coast with the exception of the southwestern portion of the United States. The red-spotted garter snake, *Thamnophis sirtalis concinnus*, is one of twelve subspecies

described and inhabits northwestern Oregon and southwestern Washington (Brown *et al.*, 1995). For *T. s. concinnus*, mating occurs post-emergence from winter dormancy in late March and early April. Females ovulate in May or early June. Gestation is typically six to eight weeks with parturition of live young occurring between late July and early September.

Summary of Research and Experimental Goals

The unifying goal of these investigations was to elucidate the factors contributing to sexual size dimorphism observed in the red-spotted garter snake. Chapter II explores differential parental investment and the ontogeny of sexual dimorphism in this viviparous reptile. We hypothesized that the sexual dimorphism observed in *T. s. concinnus* is related to differential levels of parental investment. We predicted that adult females would contribute more energy to female offspring either by increasing the ratio of females within a clutch or by producing larger female young. This hypothesis stems from the observation that adult females of this species are the larger sex. We have found that on average female offspring are relatively larger than males. However, this differential condition is not a result of increases in the number or body size parameters (body mass and SVL) of neonatal females. In fact, as maternal size and presumably capacity for offspring increases, the number of males produced increases. Condition of offspring is unrelated to the capacity of adult females to produce young.

Chapter III explores the hormonal influences on the mediation of sexually dimorphic growth in this species. Previous work in this area has indicated a testicular

factor that contributes to the reduced size found in adult male red-spotted garter snakes. It was our goal to further identify the particular gonadal factors that play a role in differential growth. The evidence suggests that in this species the influence on growth of the sex steroids, estradiol and testosterone, is in itself sexually dimorphic.

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CHAPTER II

ALLOCATION OF PARENTAL INVESTMENT IN THE RED-SPOTTED GARTER SNAKE, *Thamnophis sirtalis concinnus*

Darren T. Lerner and Robert T. Mason

Abstract

The allocation of energy for reproduction has been examined in a diverse array of organisms. Much attention has focused on the interrelationships of parental size, offspring size, and number of offspring per reproductive bout. Although spatial and temporal variation in these variables has been investigated, these relationships have not been adequately tested while accounting for differences in offspring gender. Using regression analysis we examined the sex-specific allocation of parental investment in the red-spotted garter snake, *Thamnophis sirtalis concinnus*. These data were collected in the field and from 108 adult females that produced 782 female and 790 male offspring in the laboratory. Adult females are heavier and longer than males and the regressions of adult body mass on snout-vent length (SVL) have significantly different slopes. Contrary to adults, the relationship of offspring body mass and SVL was the same between the sexes in neonates. In addition, analysis of variance of the residuals from the unified regression of offspring body mass on SVL indicated that females are larger than males at birth. These results indicate that the full extent of sexual size dimorphism found in adults is not manifest at birth, although some dimorphism between the sexes does exist. Furthermore, we found that females switch to producing predominately male clutches at a pivotal adult size or age, which coincides with a period when offspring size is maximized.

Introduction

Reproductive effort is defined as the portion of the total energy budget that is devoted to reproduction including mating, egg production and parental care (Hirshfield and Tinkle, 1975). Parental investment is the amount of resources invested in the current reproduction of young (Wilbur, 1977). Therefore, parental investment is a component of reproductive effort that identifies the amount of energy transferred to offspring. Some authors have explored the partitioning of this particular energy component in a theoretical context. Smith and Fretwell (1974) constructed a two-dimensional graphical model to predict the energy expenditure per offspring that would maximize parental fitness. This model predicts that an increase in parental investment to offspring reduces the number of offspring that are produced. Other models predict that while the partitioning of energy between offspring number and size is optimized, variable levels of competition, predation and environmental exposure (*e.g.* temperature, precipitation) influence this optimization (Brockelman, 1975; Wilbur, 1977). Like Smith and Fretwell, these authors rely on the assumption that parental investment expenditure is based on production of young rather than on other reproductive activities such as territorial defense, courtship or parental care.

Some investigators have empirically tested the predictions made by the theoretical models. Correlation analysis has been used to examine the relationships between several parameters that are indicative of parental investment in reptilian species (*e.g.*, offspring size, clutch, brood or litter size, total clutch mass and maternal size) (Seigel and Ford, 1987). Comparative analyses of these parameters associated with parental investment vary, not only in regard to the particular factors examined, but also across species, time, habitat and reproductive mode (Congdon *et al.*, 1978; Nussbaum, 1981; Seigel and Fitch,

1985; Ferguson and Snell, 1986; Jones *et al.*, 1987; Ford and Seigel, 1989; Ferguson *et al.*, 1990; or for a general review see Seigel and Ford, 1987). Generally, total clutch mass, clutch size (hereafter referred to as the number of offspring per clutch), and offspring sizes are positively correlated with maternal size. It has been suggested, however, that indirect effects may create a negative relationship between maternal size and the size of offspring (King, 1993).

The iguanid lizards *Holbrookia maculata* and *Sceloporus undulatus* exhibit age dependent variation in clutch frequency and number of offspring per clutch (Jones *et al.*, 1987). Second clutches of multiple clutch lizards result in a decreased number of offspring per clutch and an increased egg and hatchling size for *Sceloporus undulatus* in the laboratory (Nussbaum, 1981). Similar shifts are observed in the field for *Uta stansburiana* (Ferguson and Snell, 1986). Number of offspring per clutch is reduced in reproductive seasons following subnormal levels of precipitation in both lizards and snakes (Seigel and Fitch, 1985; Ferguson *et al.*, 1990). In addition, the numbers of offspring per clutch and clutch mass (but not offspring size) vary with prey availability in viviparous snakes (Ford and Seigel, 1989). Lastly, differences between the reproductive modes of unisexual and bisexual *Cnemidophorus* species result in differential offspring size and number of offspring per clutch (Congdon *et al.*, 1978).

Investigators working with reptilian models often utilize the relationship of the total clutch mass to female post-partum body mass or relative clutch mass (RCM) as a comparative index of parental investment within and between species, but not without criticism (Hirshfield and Tinkle, 1975; Barron, 1997). A comparison within squamates indicates variability in RCM attributed to differences in body shape (Shine, 1992).

Among lizards, differences in foraging modes are associated with differences in RCM (Vitt and Congden, 1978). While viviparous snakes exhibit lower RCM's than oviparous individuals (Seigel and Fitch, 1984), there is no correlation between RCM and maternal length or age for animals of either reproductive mode (Seigel *et al.*, 1986). These investigations describe considerable variation in the effects of genetic and environmental factors on reptilian parental investment and its products, but have not differentiated between male and female offspring.

Fisher (1930) predicted selection for equal investment of male and female young due to equivalent costs of production of each sex. Termed sex ratio theory, this states that parental fitness would be maximized by an equal expenditure of energy in young (Fisher, 1930; see also McGinley *et al.*, 1987). However, it has been hypothesized for mammals and birds, of which males are typically the larger sex, that natural selection favors decreases in the sex ratio of males to females as maternal condition decreases (Trivers and Willard, 1973). Data from a number of species support this hypothesis (Trivers, 1985).

Few investigators have examined intraclutch variation in reptiles with regard to parental investment. Parental investment per progeny (relative mass per offspring) decreases with maternal age for the garter snake *Thamnophis butleri*, but differences between male and female offspring were not examined (Ford and Killebrew, 1983). A study of the redbelly snake, *Storeria occipitomaculata*, found no evidence for intraclutch variation in offspring size (Brodie and Ducey, 1989). Dunlap and Lang (1990) found that the sex ratio of males increased with maternal size in *Thamnophis sirtalis sirtalis*. Yet in

the marine snake *Enhydrina schistosa*, as maternal size increases female offspring size and number increase (Lemen and Voris, 1981).

The purpose of this study was to explore differential parental investment and the ontogeny of sexual dimorphism in a viviparous snake. We hypothesized that the sexual dimorphism observed in adult *T. s. concinnus* is related to differential levels of parental investment favoring female offspring. Similar to the increases observed in mammals and birds where males are the larger sex, we predicted that adult females would contribute more energy to female offspring by increasing the ratio of females within a clutch, producing larger female young or both.

Materials and Methods

Animal Collection and Husbandry

Gravid *T. s. concinnus* (n = 108) were collected in June and July of 1995, 1996, and 1997 from various sites within Benton County, Oregon. All adult animals were returned to their respective field sites within a few weeks of parturition (generally throughout August and early September).

Adult females were maintained in 10-gallon glass aquaria with a paper substrate (Animal Specialties Inc., Hubbard, OR). Air temperature in the room ranged from 17-28°C with an additional midday increase from 28-33°C provided to individual aquaria by a 25-watt soft white light bulb. The daily light:dark cycle was held constant at 14L:10D. Relative humidity ranged from 55-65%. All gravid females were measured from snout-

to-vent (SVL) to the nearest millimeter by gently stretching them along a meter stick until they relaxed and were weighed to the nearest tenth of a gram upon capture and within twenty-four hours post-partum. Individuals were offered 3-6 mosquitofish (*Gambusia* spp.) and earthworms (*Lumbricus* spp.) each week and provided water *ad libitum*. We identified the gender of individual newborns by inspection of the urogenital opening and careful eversion of hemipenes. Neonates were measured and weighed in similar fashion as adults within twenty-four hours of birth.

Statistical Analyses

Heterogeneous data were natural log-transformed whenever necessary to meet the assumptions of normality. Pearson product moment correlations were used to examine the relationships between parental investment parameters. Tests for heterogeneity of slopes both between years and between sexes were constructed using a general linear model (SAS Institute Inc.). Means of heteroscedastic variables were compared by a least squares means test. Means of homoscedastic variables were analyzed by comparison of the residuals from a linear regression (ANOVA). Residual analysis was utilized to examine overall body size (hereafter referred to as body size/condition) between the sexes when statistically plausible. Means were used for comparisons of body mass and SVL within sexes to satisfy assumptions of independence (ANCOVA). For between sex comparisons of offspring the assumption of independence was maintained by ANOVA of indexed absolute differences between sex-specific dependent variables.

Results

Adults

Body mass of adult males and nongravid females was positively correlated with SVL (females: $r = 0.92$, $P < 0.001$, and males: $r = 0.92$, $P < 0.001$). Regression of body mass on SVL had a significantly greater slope in females ($P < 0.001$, $F = 17.48$) (Fig. II.1). Non-gravid females were heavier ($P < 0.001$, $T = 12.06$) and longer ($P < 0.001$, $T = 8.97$) than males.

Post-partum females

Female post-partum (hereafter referred to as maternal) body mass was positively correlated with SVL for each year of the study. There were no significant differences between the slopes of these regressions ($P = 0.569$, $F = 0.57$). Analysis of the residuals from the single regression of body mass on SVL in the combined data set ($r = 0.93$, $P < 0.001$) indicated no significant difference in body size/condition among post-partum females ($P = 0.469$, $F = 0.76$).

Number of Offspring per Clutch

Total number of offspring per clutch (male and female offspring combined) was positively correlated with maternal SVL ($r = 0.50$, $P < 0.001$) (Fig II.2). There was no significant difference in this relationship among years ($P = 0.451$, $F = 0.80$). Total number of offspring per clutch ranged from 5-34, averaged 14.3 ($n = 108$, $SE = 0.5$) and was not significantly different between years ($P = 0.740$, $F = 0.30$). Total number of offspring per clutch was not correlated with maternal body size/condition ($r = -0.08$, $P = 0.388$).

Number of male offspring per clutch and maternal SVL were positively correlated for each year of the study. There were no significant differences between the slopes of these regressions ($P = 0.099$, $F = 2.37$). There were no significant differences in the number of male offspring per clutch between years after controlling for differences in maternal SVL ($P = 0.524$, $F = 0.65$).

Similarly, the number of female offspring was positively correlated with maternal SVL in 1996 and 1997, but not in 1995 (1995: $r = 0.37$, $P = 0.224$; 1996: $r = 0.48$, $P = 0.002$; 1997: $r = 0.335$, $P = 0.013$). However, the slopes of these regressions proved to be homogeneous with ($P = 0.923$, $F = 0.08$) or without ($P = 0.798$, $F = 0.07$) the inclusion of the 1995 data set. There were no significant differences in the number of female offspring per clutch between years after adjusting for differences in maternal SVL (1995 vs. 1996: $P = 0.617$, 1995 vs. 1997: $P = 0.857$, 1996 vs. 1997: $P = 0.601$, from a least squares means test).

Number of offspring per clutch as a function of maternal SVL was significantly different between the sexes ($P < 0.001$, $F = 14.60$) (Fig II.3). There was no significant difference in the overall number of male or female offspring after allowing for differences in maternal SVL ($P = 0.735$, $T = 0.34$, from a least square means test).

Relative Clutch Mass

Regressions of the total clutch mass (male and female offspring combined) with maternal body mass for each year revealed a positive correlation between the variables ($r = 0.62$, $P < 0.001$) (Fig. II.4). There were no significant differences between the slopes of these regressions, ($P = 0.231$, $F = 1.48$). There were no significant differences in mean total clutch mass between years ($P = 0.538$, $F = 0.62$). For the three years combined, total clutch mass was not correlated with maternal body size/condition ($r = 0.03$, $P = 0.756$).

Male clutch mass and maternal body mass were positively correlated for each year of the study. There were no significant differences between the slopes of these regressions, ($P = 0.238$; $F = 1.45$). There were no significant differences in mean male clutch mass between years after controlling for differences in maternal body mass ($P = 0.539$, $F = 0.62$).

Similar to the number of female offspring per clutch, female clutch mass was positively correlated with maternal body mass in 1996 and 1997, but not in 1995 (1995: $r = 0.36$, $P = 0.258$; 1996: $r = 0.66$, $P < 0.001$; 1997: $r = 0.40$, $P = 0.002$). However, a comparison of the slopes of these regressions proved to be homogeneous

with ($P = 0.192$, $F = 1.68$), or without ($P = 0.561$, $F = 3.74$), the inclusion of the 1995 data set. There were no significant differences in mean female clutch mass between years after controlling for differences in maternal body mass ($P = 0.906$, $F = 0.10$).

Clutch mass, as a function of maternal body mass, was significantly different between the sexes ($P < 0.001$, $F = 16.87$) (Fig. II.5). There was no significant difference in mean clutch mass between males and females after allowing for differences in maternal body mass ($P = 0.916$, from a least square means test).

Total mass of the clutch (males and females combined) was positively correlated with the total number of offspring produced ($r = 0.89$, $P < 0.001$) (Fig. II.6). However, neither the average mass per offspring or offspring condition could be predicted from the total number of offspring produced ($r = 0.08$, $P = 0.408$) and (males: $r = 0.04$, $P = 0.651$, and females: $r = 0.03$, $P = 0.975$).

Offspring

Body mass was positively correlated with SVL in male offspring for each year of the study. A test of the heterogeneity of slopes revealed a significant difference in the relationship of body mass to SVL between years ($P = 0.008$, $F = 7.65$). This difference was attributed to the 1995 data set. A comparison without this data set yielded no significant difference between slopes of the remaining years ($P = 0.351$, $F = 0.88$).

There was no significant difference in mean body mass after adjusting for differences in SVL between 1995 and 1996 ($P = 0.691$, $T = 0.40$) or between 1995 and 1997 ($P = 0.861$, $T = 0.18$) from least square means tests. There was no significant difference in the residuals representing body size/condition between 1996 and 1997 ($P = 0.113$, $F = 2.04$).

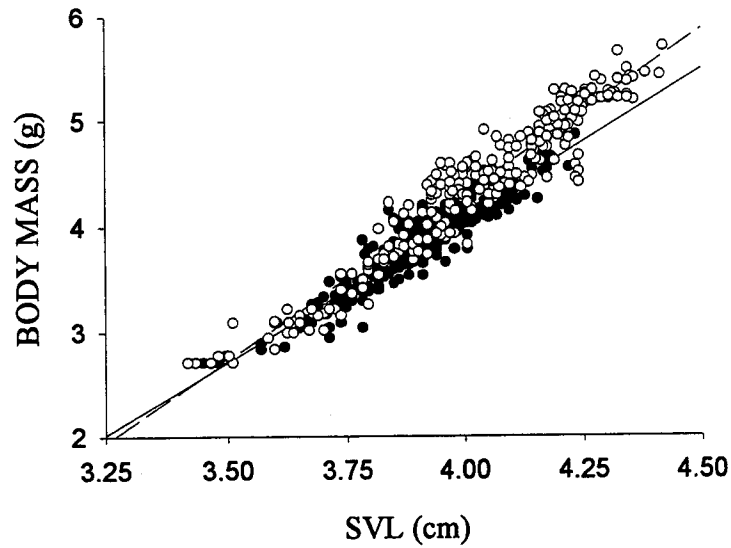


Fig. II.1. Relationship of adult body mass and SVL. The regression equation for females ($n = 246$) is $y = -8.2659 + 3.1406x$ (open circles). For males ($n = 269$) $y = -7.0067 + 2.7757x$ (closed circles). Data are natural log-transformed.

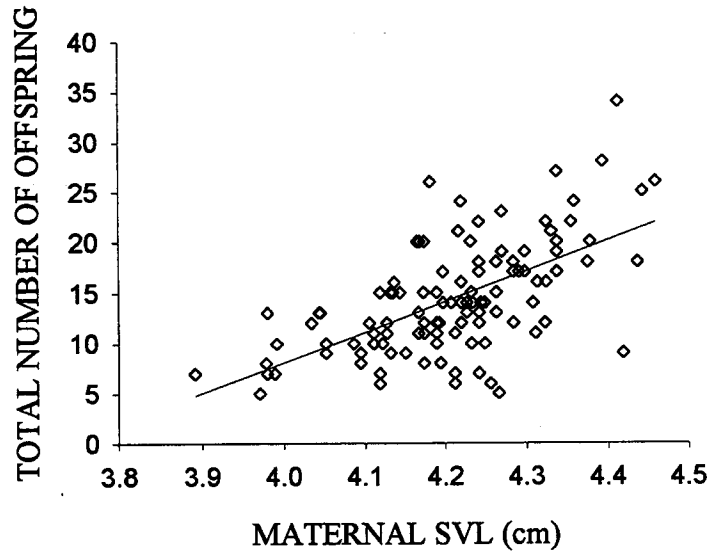


Fig. II.2. Relationship of the total number of offspring and maternal SVL (natural log-transformed) The regression equation is $y = -112.617 + 30.173x$.

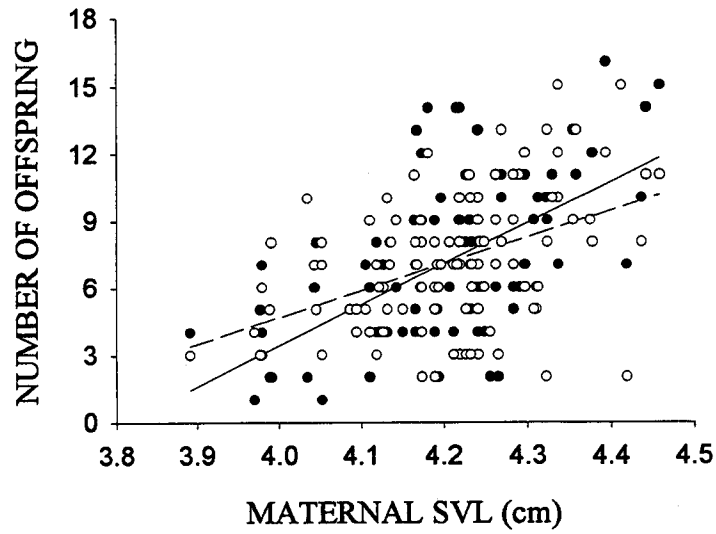


Fig. II.3. Relationship of the sex-specific number of offspring and maternal SVL (natural log-transformed). Females ($y = -69.618 + 18.258x$) are depicted by open circles and a broken line. Males ($y = -42.999 + 11.916x$) are depicted by closed circles and a solid line.

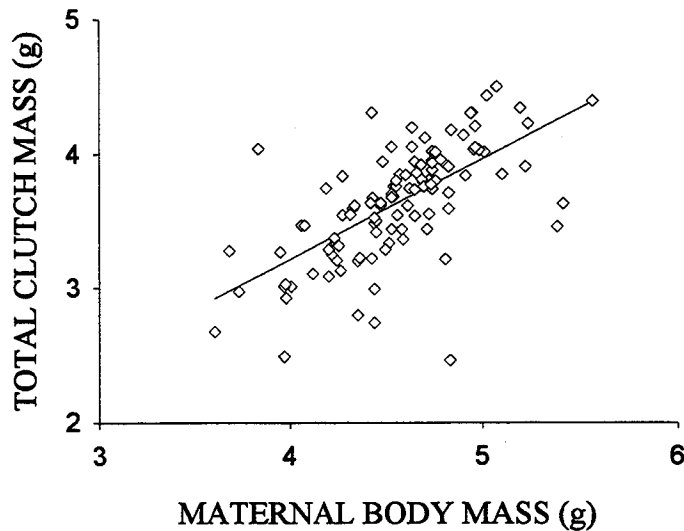


Fig. II.4. Relationship of the total clutch mass and maternal body mass (log-log scale). The regression equation is $y = 0.2086 + 0.7508x$.

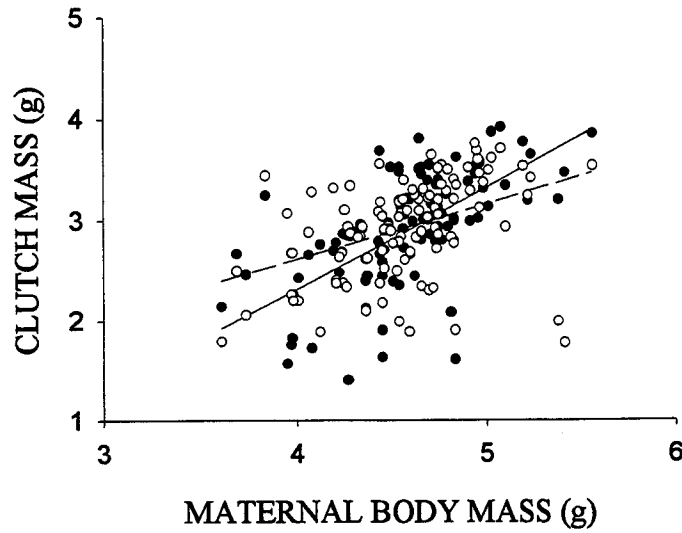


Fig. II.5. Relationship of clutch mass and maternal body mass (log-log scale). Females ($y = 0.4326 + 0.5443x$) are depicted by open circles and a broken line. Males ($y = -1.6899 + 1.001x$) are depicted by closed circles and a solid line.

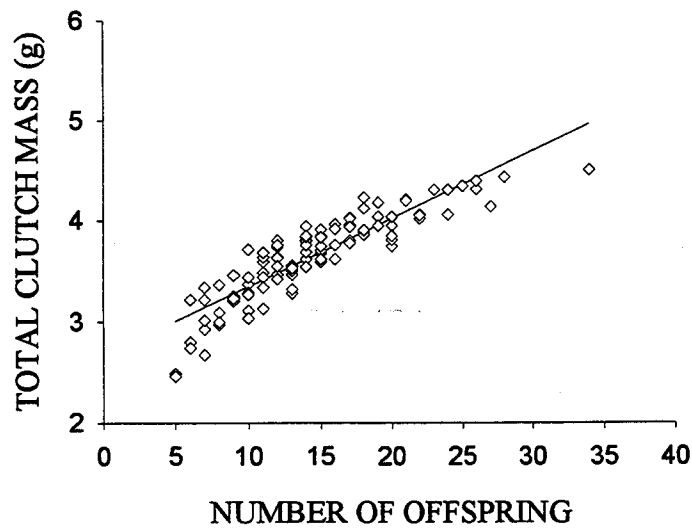


Fig. II.6. Relationship of the total clutch mass (log-transformed) to the total number of offspring produced. The regression equation is $y = 2.668 + 0.0675x$.

Female body mass was positively correlated with SVL for each year of the study. There were no significant differences between years ($P = 0.986$, $F = 0.01$). Likewise, there were no significant differences between years in the residuals representing body size/condition ($p = 0.104$, $F = 1.88$).

Relationships of body mass as a function of SVL (body composition) was homogeneous between male and female offspring ($P = 0.923$, $F = 0.01$) (Fig. II.7). Residuals from the combined regression indicated that the average female body size/condition was greater than males ($P < 0.001$, $F = 6.48$).

In addition, the separate offspring size parameters (body mass and SVL) increased as maternal body mass and SVL increased (for body mass of males: $r = 0.43$, $P < 0.001$; females: $r = 0.40$, $P < 0.001$) and (for SVL of males and females: $r = 0.34$, $P < 0.001$). Neither of these relationships were significantly different between male and female offspring (body mass: $P = 0.821$, $F = 0.05$) (Fig. II.8) and (SVL: $P = 0.998$, $F = 0.01$) (Fig II.9)

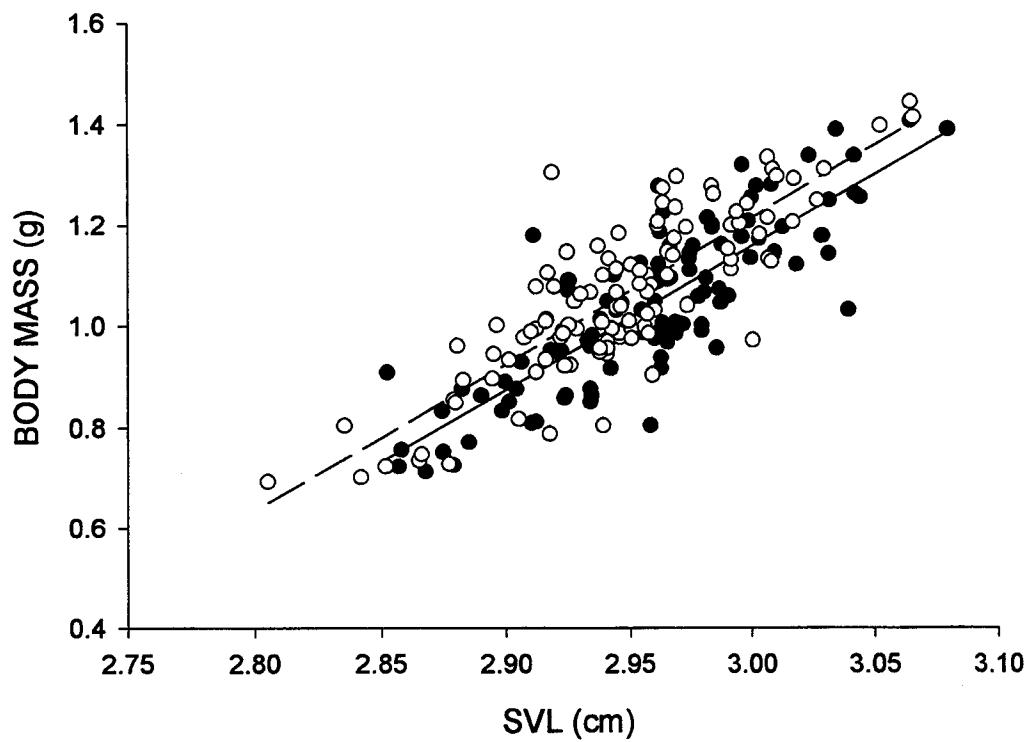


Fig. II.7. Relationship of offspring body mass and SVL (log-log scale) at birth. Females ($y = -7.4322 + 2.881x$) are represented by open circles and a broken line. Males ($y = -7.326 + 2.8272x$) are represented by closed circles and a solid line.

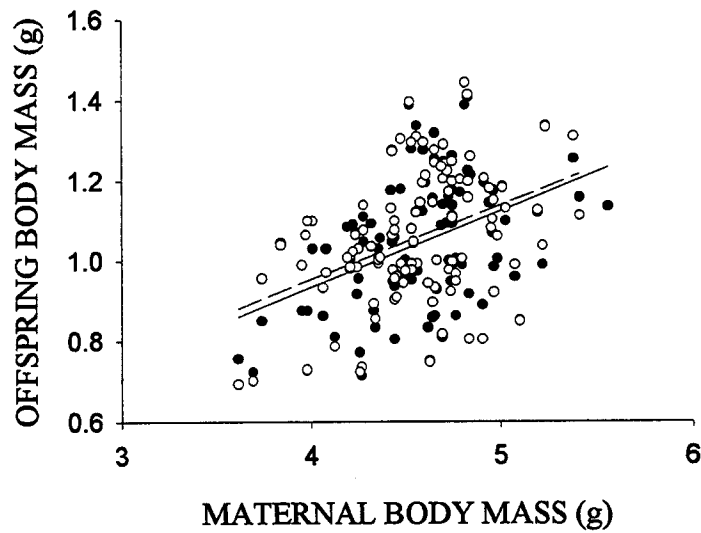


Fig. II.8. Log-log relationship of offspring body mass (clutch means) and maternal body mass. Males are represented by closed circles and a solid line ($y = 0.1767 + 0.1895x$). Females are represented by open circles and a broken line ($y = 0.2497 + 0.1852x$).

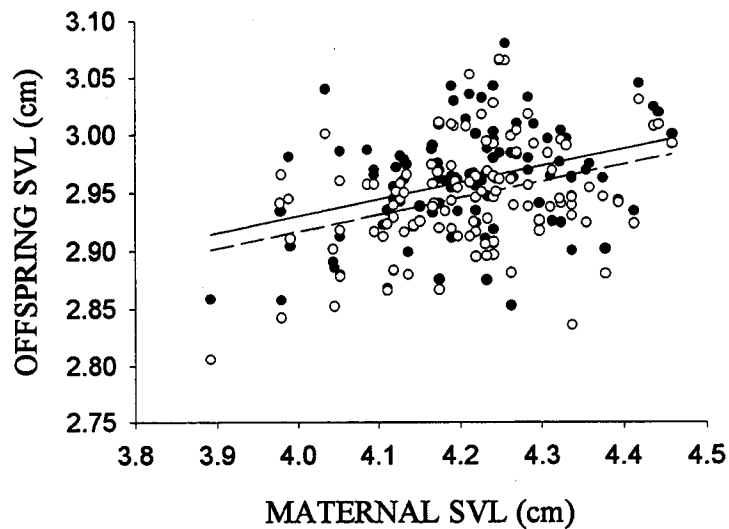


Fig. II.9. Log-log relationship of offspring SVL (clutch means) and maternal SVL. Males are represented by closed circles and a solid line ($y = 2.355 + 0.1437x$). Females are represented by open circles and a broken line ($y = 2.341 + 0.1438x$).

Discussion

Parental Investment

As the literature on reptiles suggests, many of the indicators of parental investment (*e.g.*, total number of offspring, total mass of the clutch) increase with increases in maternal size (Figs. II.2, II.4). In addition, we have found that offspring size increases with increases in maternal size (Figs. II.8, II.9). Results from these investigations disagree with the predictions of the optimal offspring size models (Smith and Fretwell, 1974; Brockelmann, 1975; Wilbur, 1977). Contrary to these models, *T. sirtalis concinnus* does not exhibit a trade off between the average mass per offspring and the number of individuals produced. Likewise, offspring body size/condition is unaffected by an increase in the number of individuals produced. This pattern remains whether we discriminated between the sexes or not. Therefore, larger adult females produce more individuals, which results in equal increases in the size of both sexes.

Sex Ratio Theory

There is a central point in the variation of maternal size (as indicated by body mass or SVL, which are highly correlated) at which an equal number of males and females are produced. An adult female below this average produces a greater number of female offspring and above yields an increased number of males (Fig. II.3).

A major assumption of the Trivers/Willard hypothesis (1973) is that differences in adult size affect male reproductive success more strongly than they affect female reproductive success. Under these assumptions, a larger adult female who produces a son will increase her number of progeny over the same size female that produces a daughter. An adult female on the smaller end of the continuum, who produces a daughter will increase her number of progeny over the same size female that produces a son (Trivers and Willard, 1973).

Our findings that the sex ratio of males increases, as adult female size increases, and that a positive correlation exists between offspring size and maternal size agrees with the Trivers/Willard hypothesis (Figs. II.3, II.5). However the hypothesis set forth by Trivers and Willard (1973) and data supporting this hypothesis (Trivers, 1985) are from mammal and bird species where males are the larger sex.

Our data indicate that adult females increase the production of male offspring in a reptile in which females are the larger sex in adults. In *T. s. sirtalis*, Dunlap and Lang (1990) also found that the male ratio increased with maternal size. Their results were based on 35 females from one reproductive season and therefore may be confounded by seasonal variation. Furthermore, offspring were obtained as advanced stage embryos, which does not accurately reflect the number of individuals surviving through birth. However, as Dunlap and Lang (1990) indicate, their methodology may avoid the possible confounding factor of differential mortality (Burger and Zappalorti, 1988).

Male garter snakes are exposed to competition for mates (Gillingham, 1987) and in *T. s. parietalis*, competition is extreme (Crews and Garstka, 1982). Joy and Crews (1988) found that male size (body mass or SVL) was not a significant component of

mating success at the over-wintering dens in this Canadian population of garter snakes. In two years at the same site, Shine *et al.* (in preparation) found that larger males, as indicated by body mass, SVL or tail length, exhibited greater mating success in mating balls outside the dens. In addition, the number of males participating did not have an effect on the success of larger males. Therefore, in extreme environments size may play a role in competition for mates regardless of the number of males competing.

The primary sex ratio of offspring born in our study was approximately 0.50 (782 females and 790 males). This is not significantly different from expected if sex is genetically determined. While individual variation in the sex ratio is likely influenced by the capacity of a female to invest in her offspring, variable environmental temperatures may play a role. Dunlap and Lang (1990) reanalyzed data from Bull (1980) and found that gravid *Nerodia fasciata* subjected to relatively high temperatures increased the ratio of female offspring. Clutches from females subjected to relatively low temperatures yielded predominately male clutches. While there is extensive laboratory and field evidence indicating a role for temperature in determining the sex of offspring (TSD) in crocodilians, many turtles and some lizards (Bull, 1980; Bull 1983), the influence of TSD on the sex ratio in *T. s. concinnus* has not been examined.

Growth in most reptiles, including snakes, is indeterminate (Fitch, 1965; Porter, 1972). Therefore variation in size of reproductively mature females may reflect variation in age. We conclude that as maternal size increases with age, selection favors the production of male biased clutches. At a pivotal adult size or age, females switch to producing predominately male clutches, which coincides with a time when offspring size is maximized.

Because male size may play a role in reproductive success, competition for mates may play a role in these selective forces. Also, we cannot rule out a role for temperature or differential mortality as possible factors shaping variable sex ratios.

Sexual Size Dimorphism

In accordance with the literature, adult females of this species are longer and heavier than males. It is important to recognize that the observed dimorphism is not merely based on differences in weight and length, but in the relationship of weight as a function of length or body composition between the sexes (Fig. II.1). With respect to this relative size, adult males and females can be considered distinct populations.

To account for the major parameters indicative of size in snakes, we used the residuals from regressions of body mass on SVL (King, 1993; Barron, 1997). At birth, female offspring are larger than males, but the relationship of body mass to SVL, which does not exhibit significant variation within the sexes, is homogeneous. (Fig. II.7).

Some investigators suggest that variation in parental investment resulting in significant variance in propagule, egg or offspring size of amphibians may provide increased fitness in variable environments (Crump, 1981; Kaplan, 1980; Kaplan and Cooper, 1984). Most studies analyzing variation in offspring size of reptiles use body mass or SVL to represent body size (*e.g.*, Dmi'el, 1967; Shine, 1978; Ford and Killebrew, 1983; Scudder-Davis and Burghardt, 1987; Brodie and Ducey, 1989; King, 1993; Gregory and Prelypchan, 1994). Working with these body size parameters, some authors have examined variation in offspring size but have not differentiated between males and

females (Dmi'el, 1967; Shine, 1978; Brodie and Ducey, 1989; King, 1993). Others have found no difference in body mass or SVL between males and females at birth (Ford and Killebrew, 1983; Scudder-Davis and Burghardt, 1987; Gregory and Prelypchan, 1994).

While some degree of sexual dimorphic character exists at birth, the extent is not equal to that found in adults. This evidence suggests other differences must exist and/or occur between ontogeny and sexual maturity to produce the full extent of the observed dimorphism. Differential body composition may contribute to differences in life history traits thus influencing foraging tactics, competition, and mating behavior (Shine, 1993). Whether the source of variation found among neonates is solely attributed to differential maternal investment or manipulated by the embryonic development of individual offspring requires further study. While environmental cues (*e.g.*, temperature, precipitation) may also play a role, it has been suggested by Crews *et al.*, (1985) that differential growth caused by testicular hormones in males may be a contributing factor. Investigations based on parental investment from the same individuals over time would be highly informative.

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Chapter III

THE INFLUENCE OF SEX STEROIDS ON SEXUAL SIZE DIMORPHISM IN THE RED-SPOTTED GARTER SNAKE, *Thamnophis sirtalis concinnus*

Darren T. Lerner and Robert T. Mason

Abstract

Some investigators have attributed sexual size dimorphism, which is common in many animals, to the regulation of growth by gonadal hormones experienced early in life. It has been hypothesized that small male body size in garter snakes may be determined by testicular factors. The red-spotted garter snake, *Thamnophis sirtalis concinnus*, exhibits adult sexual size dimorphism such that females are the larger sex. To begin to understand which endogenous hormones may influence growth in this species we constructed growth curves and hormone profiles of circulating levels of the two predominant gonadal steroids, estradiol-17 β (E₂) and testosterone (T). In addition we manipulated growth with the use of exogenous hormones and hormone antagonists. Gravid females were collected and housed in the laboratory until parturition for three consecutive years beginning in 1995. Changes in body mass and snout-vent length (SVL) were recorded from individuals born in the laboratory in 1995. From individuals born in 1996 we constructed profiles of circulating plasma levels of E₂ and T, which were measured by radioimmunoassay following chromatographic separation on celite columns. Neonates born in the laboratory in 1997 received Silastic capsules containing crystalline E₂, T, antiandrogen (cyproterone acetate), antiestrogen (tamoxifen) or a blank capsule at one or ten weeks of age. Body mass and SVL growth curves were significantly different beginning at either 20 or 24 weeks of age respectively. Circulating levels of E₂ and T were present in males and females from birth through 15 weeks and at 36 weeks of age. Age did not have a significant effect on levels of sex steroids in either sex. In addition, there were no significant differences in the mean values of E₂ or T between the sexes.

Within treatment comparisons indicated that there was no age effect associated with the timing of implantation and growth. Differences in the SVL growth curves of the treated and untreated snakes were significant after 24 weeks of age. Antiestrogen produced male-like growth in females, but had no effect on males. Antiandrogen had no effect on either males or females. Exogenous T reduced female growth to that observed in males and E_2 reduced male growth compared to untreated males. Our results suggest that a basal level of either E_2 or T is sufficient in males to retain the male condition. Similar endogenous levels of E_2 appear to have growth promoting effects in females. Circulating plasma levels of T do not appear to play a role in female growth. We discuss these differential effects of sex steroids, as well as the potential for genetic and environmental influences on sexual size dimorphism in this species.

Introduction

Sexual dimorphism associated with differential body size in reptiles may contribute to differences in life-history traits thus influencing foraging tactics, competition, and mating behavior (Shine, 1993). Focusing on proximate mechanisms, investigators attribute sexual size dimorphism to the regulation of growth by gonadal hormones experienced early in life. Testosterone (T) increases growth of males in some mammalian and avian models (Slob *et al.*, 1975; Gentry and Wade, 1976; Grey *et al.*, 1979; Czaja, 1984; Cikos *et al.*, 1992; Peralta *et al.*, 1994) while decreasing growth in others (Swanson, 1967; Bubenik *et al.*, 1975; Fennell and Scanes, 1992).

In rats, differential growth rates from puberty onward contribute to the larger size of adult males. A rise of circulating androgens in males at a critical period has been shown to play a role in increased growth (Slob *et al.*, 1975). In guinea pigs, the larger size of males stems from significant differences in the activational effects of testosterone on body size (Czaja, 1984). Contrary to the size differences observed between male and female rats and guinea pigs, adult female golden hamsters are longer and heavier than males. Gonadectomy of neonatal male golden hamsters increases growth rates to the levels observed in females (Swanson, 1967). It would appear that the role of T in males of species where males are the larger sex is to increase growth and to decrease growth in males where females are larger.

Much of the work associated with hormonal influences on growth has been conducted with mammals, birds and fish. It has been hypothesized that small male body size in some reptiles may also depend on testicular factors (Crews *et al.*, 1985). These

investigators observed that circulating levels of testosterone in male red-sided garter snakes, *Thamnophis sirtalis parietalis*, increase markedly by the third week of life (compared to the first and second weeks only). Growth rates of neonatal females exceeded males beginning at approximately ten weeks of age. Gonadectomy of neonatal male *T. s. parietalis* increases growth rates to levels exhibited by females. Because testosterone is the major androgen of the testis, a correlation has been drawn between its action and differential growth observed in males. However, this has not been tested empirically in the garter snake model.

The present study was conducted to investigate sexually dimorphic growth in the red-spotted garter snake, *Thamnophis sirtalis concinnus*. Our goal was to explore the role of gonadal hormones on sexual size dimorphism. This species exhibits adult sexual size dimorphism such that females are the larger sex. To gain an understanding of which endogenous hormones may influence growth in this species, we constructed a hormone profile of estradiol and testosterone from birth to 15 weeks and at 36 weeks of age. Often, researchers exploring the effects of gonadal hormones on growth remove the gonads, thereby removing a wide array of factors. We experimentally tested the effects of exogenous hormone as well as the removal of endogenous hormone on growth with the use of estrogen and androgen antagonists.

Materials and Methods

Animal Collection and Husbandry

Gravid *T. s. concinnus* were collected in June and July for three consecutive years beginning in 1995. Adult females were collected from various sites throughout Benton County, Oregon. All adult animals were returned to their respective field sites shortly after parturition (generally throughout August and early September).

We identified the gender of individual newborns by inspection of the urogenital opening and careful eversion of hemipenes. At birth all neonates were given an individual number by clipping the ventral scutes with a scleral punch. In addition, individuals were measured from snout-to-vent (SVL) to the nearest millimeter and weighed to the nearest tenth of a gram. Over the course of these investigations, neonates were randomly housed in 10-gallon glass aquaria with a paper substrate (Animal Specialties Inc., Hubbard, OR.) and measured once monthly. Animals born in the laboratory in 1995 were maintained at $20^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with an additional midday increase from $28\text{--}33^{\circ}\text{C}$ provided to individual aquaria by a 25-watt soft white light bulb. Air temperature for animals born in 1997 ranged from $17\text{--}28^{\circ}\text{C}$ with the same midday increase provided in 1995. The daily light-dark cycle varied relative to natural conditions. Relative humidity ranged from 55-65%. Individuals were offered mosquitofish (*Gambusia* spp.) and earthworms (*Lumbricus* spp.) once weekly *ad libitum* and provided a constant water supply. Care was taken such that all animals were individually offered food and were observed to eat.

Hormone profile

Blood samples were obtained from randomly chosen neonates such that samples from ten individual males and ten individual females were obtained each week beginning at 0 weeks (= birth) and continuing for fifteen weeks. Approximately 40-80µl of blood was collected from the caudal vein. Samples were centrifuged for three minutes at 3,000 rpm, the plasma was separated, and both the red blood cells and the plasma were stored at -80°C. All samples were obtained within 2 minutes between 0900 and 1300 hours. Plasma levels of estradiol-17β and testosterone were measured by radioimmunoassay following chromatographic separation on celite columns as described by Wingfield and Farner (1975). High-pressure liquid chromatography conducted by Carl Schreck and Grant Fiest (Oregon State University) provided additional validation of hormone levels.

Hormone manipulation

All animals were hypothermically anesthetized on wet ice (Crews *et al.*, 1985). Thirty male neonates received a 5 mm Silastic capsule (Dow Corning, Midland, MI.) (0.76 mm ID x 1.65 mm OD) containing 3 mm of packed crystalline E₂, T, antiandrogen (cyproterone acetate), antiestrogen (tamoxifen) or a blank capsule at one or ten weeks of age. Females received crystalline T, E₂, antiandrogen, antiestrogen or a blank capsule at one or ten weeks of age. Blood samples were obtained from the caudal vein of ten individuals representing each treatment group at the end of the study.

Radioimmunoassay of these samples was used to validate the effectiveness of the implants. Levels of T and E₂ in 36-week-old controls were analyzed along with the hormone profile mentioned above.

Statistical analysis

Two-way-analysis of variance was used to determine the significance of age and sex on gonadal hormones. Repeated-measures ANOVA (SAS Institute Inc.) was used to determine significant differences in the slope of log-transformed body mass and SVL growth curves and significant effects of sex.

Results

Growth 1995

Slope of the body mass curves of males and females did not differ between birth and 20 weeks (body mass: $P = 0.317$, $F = 1.08$). Slope of the SVL curves of males and females did not differ between birth and 24 weeks (SVL: $P = 0.132$, $F = 1.94$). However, both curves differed significantly between the sexes from 20 weeks (body mass) or 24 weeks (SVL) through the end of the study (48 weeks) (Body mass: $P = 0.011$, $F = 4.12$; SVL: $P = 0.012$, $F = 3.99$) (Fig. III.1).

Hormone Profiles

Testosterone levels of individual males and females ranged from 0.15 to 1.0 ng/ml over the entire profile. Age did not have a significant effect on circulating levels of testosterone in males or females (males: $P = 0.391$, $F = 1.08$; females: $P = 0.327$, $F = 1.16$). Likewise, E_2 levels in both sexes ranged from 0.15 to 1.2 ng/ml from birth through 15 weeks and at the 36th week. There was no significant effect of age on E_2 in either sex (males: $P = 0.963$, $F = 0.44$; females: $P = 0.121$, $F = 0.32$). There was no significant difference in the mean values of T or E_2 between the sexes ($P = 0.152$, $F = 2.07$) (Fig. III.2).

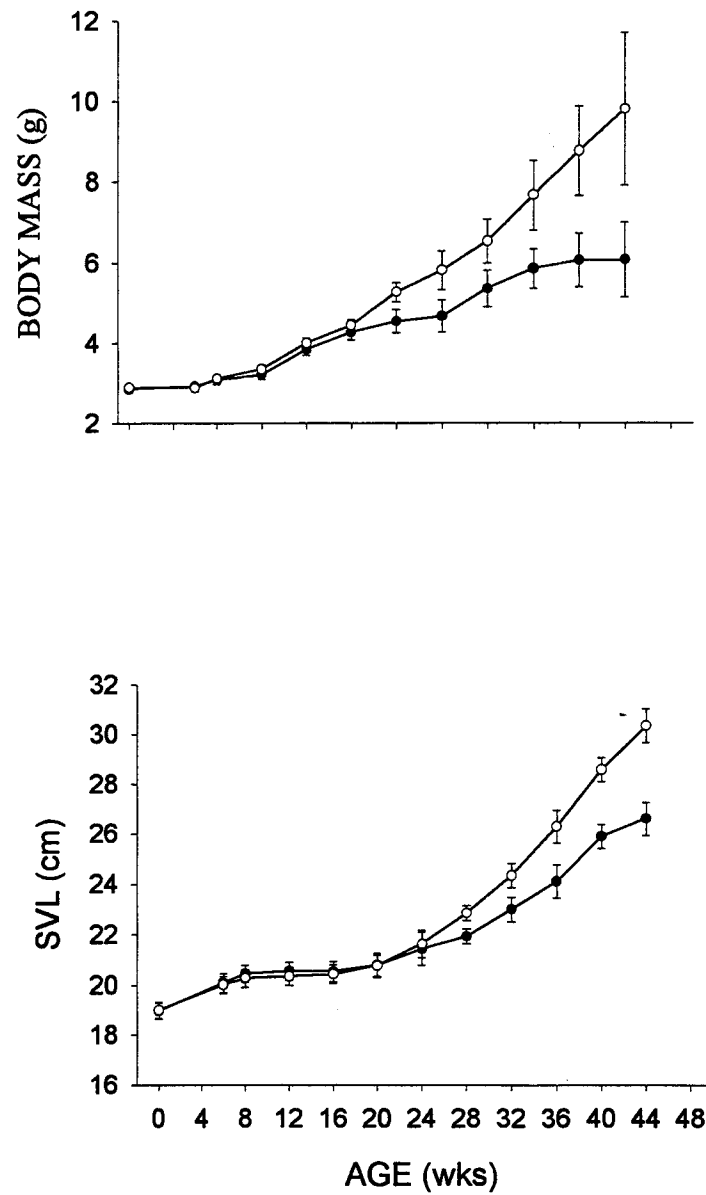


Fig. III.1. Growth of neonatal red-spotted garter snakes in 1995. Body size of snakes is presented as means \pm SE. Open circles represent females (n = 20). Closed circles represent males (n = 19).

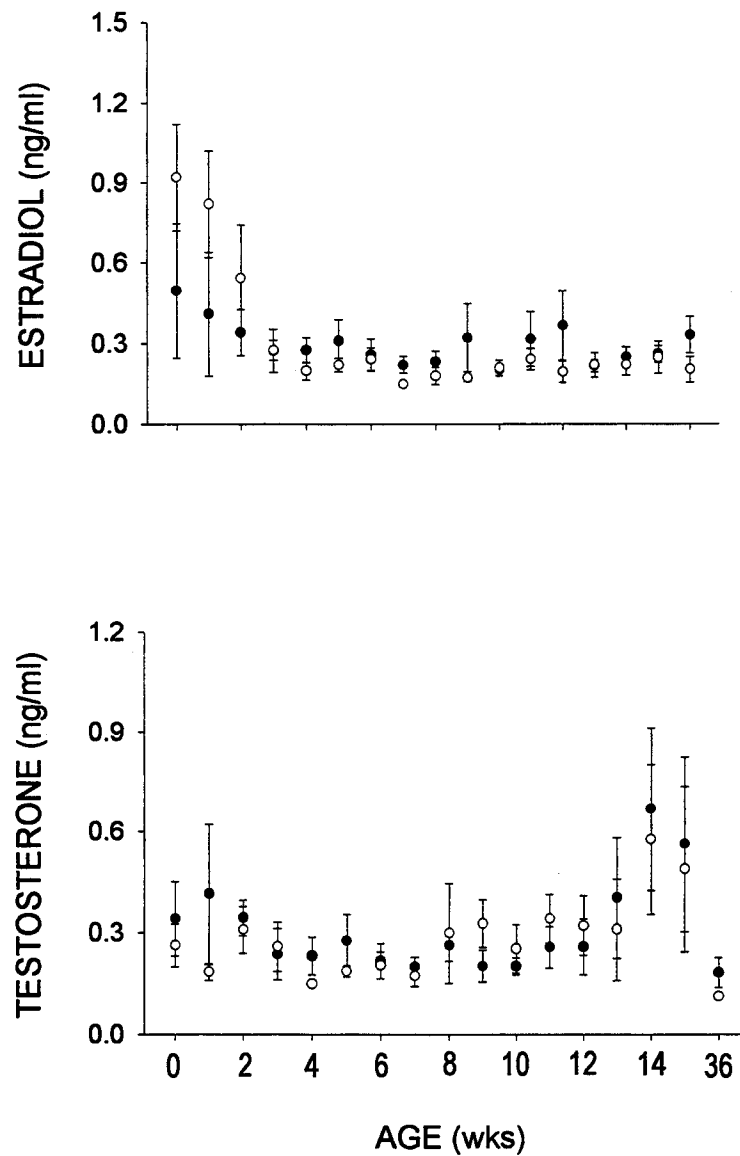


Fig. III.2. Circulating levels of endogenous E₂ and T in intact neonatal male and female red-spotted garter snakes from 0 (= birth) to 15 weeks and at the 36th week. Values are means \pm SE expressed in ng/ml. Male are represented by closed circles (n = 10 for each week) and females are represented by open circles (n = 10 for each week).

Hormonally Manipulated Growth

Within treatment comparisons indicated that there was no age effect associated with the timing of implantation and growth. Therefore all analyses of hormone treatment effects were based on combined age groups. In addition, there were no significant differences in the body mass curves between the treatment groups so the following analyses focus on changes in SVL or skeletal growth. All but one of the males implanted with testosterone died before the end of the study as did all of the females implanted with estradiol. These animals and those that did not survive the 36-week study period were removed from the analysis. Mean (\pm SE) plasma levels of T and E₂ delivered by implants was 18.62 ± 1.25 ng/ml and 22.45 ± 2.27 ng/ml, respectively.

Differences in the SVL growth curves of the treated and untreated snakes were significant beginning after 24 weeks ($P < 0.001$, $F = 3.63$) (Figs. III.3, III.4). Growth curves of male and female controls had significantly different slopes ($P < 0.001$, $F = 5.17$). Antiestrogen reduced female growth such that it did not differ significantly from that of control males ($P = 0.678$, $F = 0.18$), but was significantly lower than that of control females ($P < 0.001$, $F = 4.87$) (Fig. III.4). Growth of females treated with exogenous T was significantly less than that of control females ($P < 0.0001$, $F = 4.21$), but did not differ significantly from that of control males ($P = 0.1918$, $F = 1.91$) (Fig. III.4). Growth curves of antiandrogen and antiestrogen treated males were not significantly different from that of control males (antiandrogen: $P = 0.1063$, $F = 2.95$; antiestrogen: $P = 0.3279$, $F = 1.39$).

However, males treated with E_2 showed significantly reduced growth ($P = 0.0051$, $F = 18.53$) (Fig. III.4). Growth of females treated with antiandrogen was not significantly different from control females ($P = 0.4239$, $F = 2.01$). See Table 1 for a summary of these results.

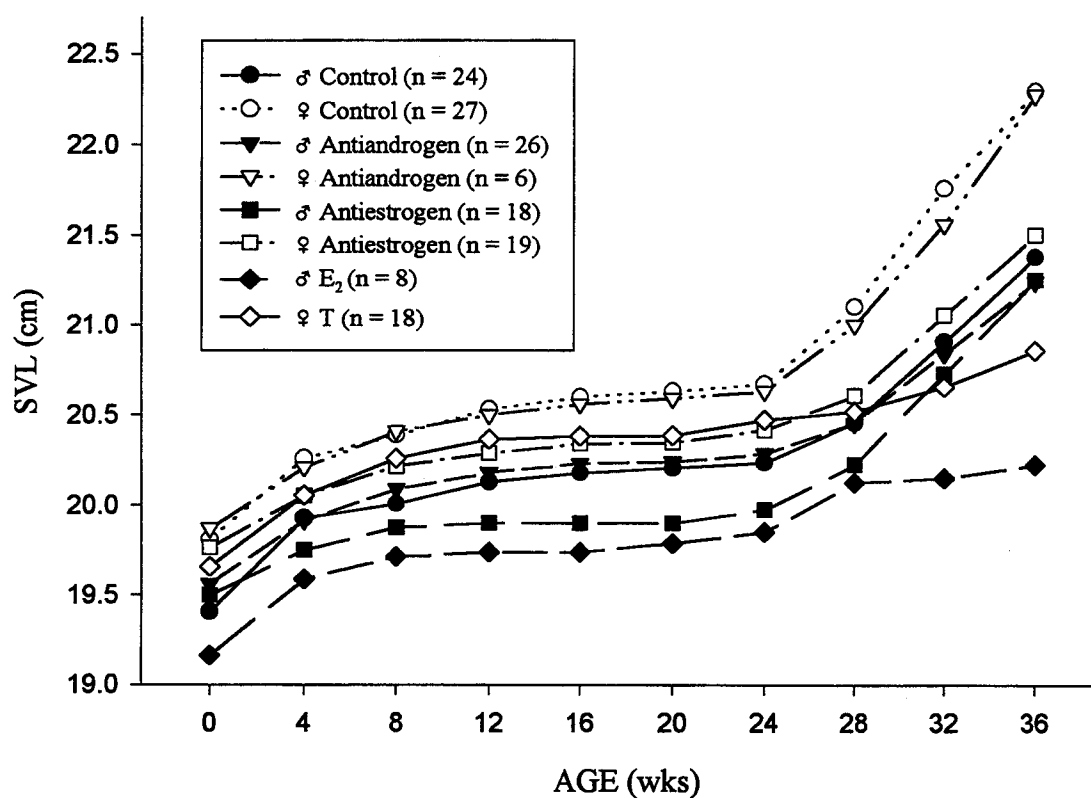


Fig. III.3. Growth of hormonally manipulated neonatal red-spotted garter snakes.

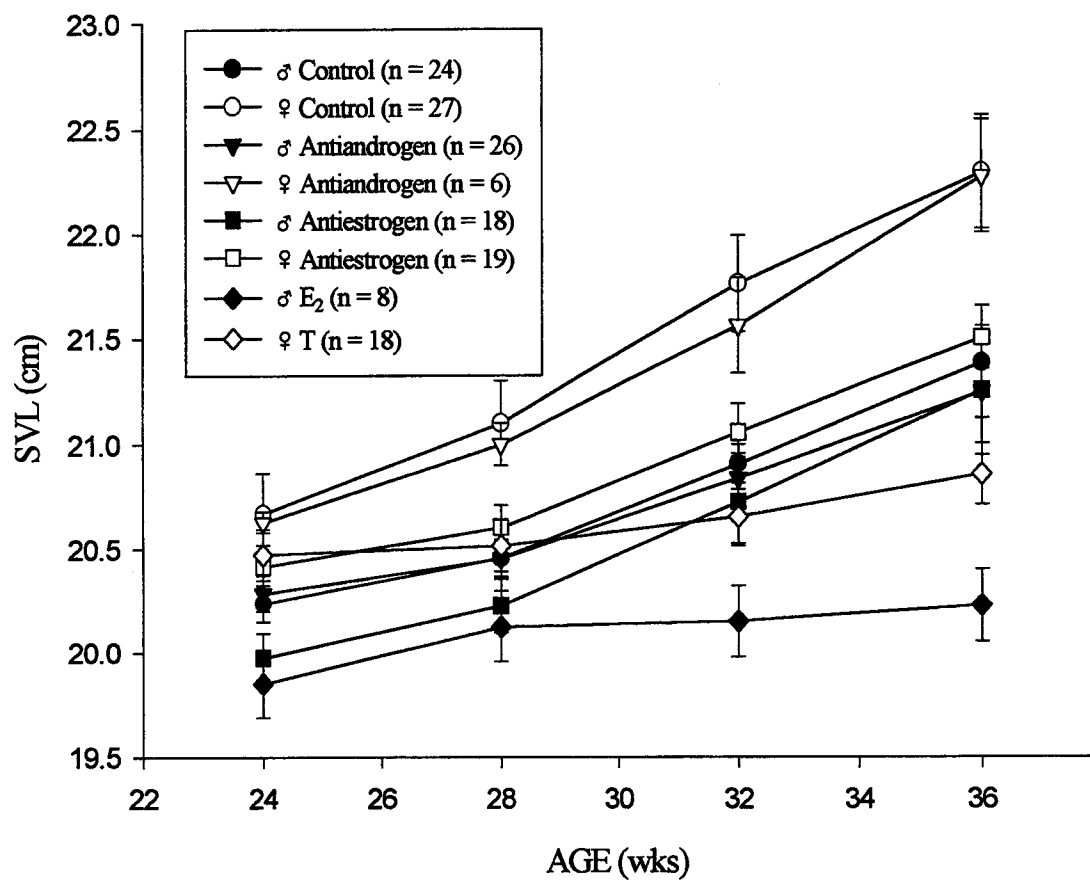


Fig. III.4. Growth of hormonally manipulated neonatal red-spotted garter snakes between 24 and 36 weeks of age. Body size is represented by mean SVL \pm SE.

Table 1. Summary of growth in hormonally manipulated neonatal red-spotted garter snakes.

Treatment	♂ Results	♀ Results
E, T	♂	♀
e, T	♂	♂
E , E, T	<♂	X
E, T, T	X	♂
E, t	♂	♀
Gonadectomized *	♀	?

Key:

E, T = endogenous hormone
E, **T** = exogenous hormone
 e, t = antiestrogen, antiandrogen

♂ = male-like growth

♀ = female-like growth

< = less than

X = deceased

* = from Crews *et al.*, (1985)

Discussion

Female SVL growth or skeletal growth was significantly greater than for males after the 24th week of life. These differences were not directly attributable to differential levels of endogenous gonadal hormones. However, antiestrogen in females reduced growth to male levels. Exogenous T had similar effects suggesting an anabolic effect of estrogens and a catabolic effect of T in females. Addition of E₂ significantly reduced male growth compared to that of untreated males, while additional T proved lethal. In addition, removal of the effects of endogenous T did not change male or female growth. Considering these findings with those of Crews *et al.* (1985) in which castrated males (removal of endogenous T and E₂) grew like untreated females, suggests that male neonatal *T. s. concinnus* need endogenous levels of either T or E₂ to maintain the male condition (Table III.1). These findings are in agreement with those in some mammals and fish where gonadal steroids have opposing effects between the sexes (Jannson *et al.*, 1985; Painson *et al.*, 1992; Holloway and Leatherland, 1997; Zou *et al.*, 1997).

One major difference between this study and the study by Crews *et al.* (1985) is the level of endogenous T found in males in that study at three weeks of age. Male *T. s. parietalis* had approximately 65 ng/ml as opposed to roughly 0.3 ng/ml at that same period in this study. However, similar levels persist in *T. s. concinnus* from ontogeny until 15 weeks and are at this level at 36 weeks of age. These levels of T and E₂ in *T. s. concinnus* are similar to those found in eight-month old snapping turtles, *Chelydra serpentina* (Rhen *et al.*, 1996).

Unlike our study, males of this turtle species exhibit significantly greater levels of T than females (Rhen *et al.*, 1996). Investigation of hormone levels at critical points of growth in other sexually dimorphic reptilian species would be highly informative.

It is possible that the antiestrogen tamoxifen may have negative effects on female growth separate from its role as an estrogen antagonist. Tamoxifen exhibits differential effects associated with different target tissues (Sato *et al.*, 1996). The most likely action of antiandrogens and antiestrogens is through competition with natural hormones for the receptor sites in the target cells (Rastogi and Chieffi, 1975). Response of target tissues to sex-steroids, antiestrogens and antiandrogens are dose dependent (Rastogi and Chieffi, 1975) and can be complex.

Similar to the findings of Crews *et al.* (1985), many of the E₂-treated males and all of the E₂-treated females (females in the Crews *et al.* study were not implanted with E₂) died before the end of the experiment. In addition, all of the males implanted with T and a considerable number of the females failed to survive. This suggests that levels of these hormones exceeding some maximal threshold may have deleterious effects.

Administration of a high dose of T reduces growth in rats, which is assumed to be caused by the aromatization of T to E₂ (Gentry and Wade, 1976; Gray *et al.*, 1979). Addition of exogenous T in male garter snakes could be converted to E₂. This would explain the reduced growth rates observed early in the growth profile of *T.s. concinnus*, but does not provide an explanation for the failure of T implanted males to survive.

A number of investigators have explored interactions between gonadal hormones and growth hormone (GH). Growth hormone is known to promote muscle and skeletal growth in animals by directly acting on target tissues as well as indirectly through

insulin-like growth factors (IGFs) (Florini *et al.*, 1996). Administration of exogenous E₂ increases GH levels in rainbow trout (Holloway and Leatherland, 1997) and female goldfish (Zou *et al.*, 1997). These GH promoting effects of E₂ are similar to those found in rat and human cell cultures (Slootweg *et al.*, 1997). In gonadectomized rats, replacement of E₂ increases GH hormone secretion in females (Painson *et al.*, 1992). Similar results are found in gonadectomized males given T (Jansson *et al.*, 1985; Jansson and Frohman, 1987). Exposure to the gonadal steroid of the opposite sex reverses these effects (Jansson *et al.*, 1985; Painson *et al.*, 1992). In general, it appears that E₂ can have growth promoting effects, but these effects may be different between the sexes.

There appears to be three distinct stages within the first year of growth in the red-spotted garter snake. Stage I occurs in the first post-natal month, during which neonates exhibit non-differential growth (Fig. 3). This growth has been attributed to metabolism of yolk reserves (Fitch, 1965; Platt, 1969; Clark 1970; Ewert, 1979). During stage II (4-24 weeks), growth rates of males and females are highly reduced, but remain undifferentiated. This period coincides with the first season of life previous to and during winter dormancy. Individuals subjected to artificial winter dormancy in the laboratory (4°C, 0L:24D and denied food) and those held at 20°C, 12L:12D and provided food, do not exhibit significant differences in growth rates during this second stage (Lerner and Mason, in preparation). Thus, reduction in growth occurs regardless of hormone treatment or food provision. Differential growth occurs during the third stage after the time coinciding with winter dormancy in nature. It would appear that neonatal growth in these animals, at least in the first year, is physiologically fixed. Further investigation of this phenomenon would be instructive.

In reptiles, females grow faster in species exhibiting larger adult females, whereas, males grow faster in species where adult males are larger (Andrews, 1982). The study of body size variation leads to the examination of influential factors manipulating rates of growth and development between and within species. The occurrence of differential growth may have important impacts on life history and ecology. Variable growth rates, which can result in significantly different adult body size, can have consequences associated with reproductive output, mating success, competition and survivorship. Understanding these adaptive qualities associated with variable growth and development necessitates a comprehension of the physiological and environmental factors involved.

Estimates of growth rates are often calculated from seasonal body size distributions obtained by mark and recapture and not from repeated measures since birth. Dimorphism in size may reflect bimaturism or a difference in age at maturity between the sexes, with the later maturing sex achieving larger body sizes (Kozlowski, 1989). In fact, there are many examples of males that mature earlier than females in snakes (Parker and Plummer, 1987). Knowledge of individual age is crucial to our understanding of sexually dimorphic growth and its impact on the behavior and ecology of animals.

Sexual size dimorphism may be influenced by completely separate factors with respect to males and females. It is possible that sexual dimorphism is the product of non-adaptive factors as reviewed by Fairbairn (1990). While we have found differences with regard to the effect of gonadal hormones, their influence on sexual size dimorphism are not necessarily adaptive and may be incidental (Lande, 1980).

Our study has shown that basal levels of endogenous E₂ and T differentially affect male and female red-spotted garter snakes. Endogenous E₂ is necessary for female-like growth. Endogenous E₂ or T is sufficient for the relatively reduced growth in males. Male-like growth can be induced in females by addition of exogenous T. We suggest that these mechanisms operate through differential feedback effects of sex steroids on the secretion of growth hormone. Analysis of growth in ovariectomized females would further define the role of these sex steroids in mediating sexual dimorphism. Further investigations into differential growth of reptiles warrant explorations of endogenous growth hormone levels and sex steroid-growth hormone interactions. Research of potential differences in sex steroid binding sites and affinity within the hypothalamus and pituitary would be informative.

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CHAPTER IV: CONCLUSIONS

Summary

We have explored the effects of parental investment as well as the influence of gonadal hormones during post-natal development on the ontogeny and mediation of sexual size dimorphism in the red-spotted garter snake. We found that while adult red-spotted garter snakes are sexually dimorphic in size, the full extent of this dimorphism is not evident at birth. Taking into account the dual nature of size in snakes (mass and length) reveals that females are larger than males at birth, but the relationship of body mass to SVL is the same between the sexes. In addition selective forces may play a role in variation of the sex ratio per reproductive event in relation to maternal size.

Estimates of growth rates are often calculated from seasonal body size distributions obtained by mark and recapture and not from repeated measures since birth. Dimorphism in size may often reflect bimaturation or a difference in age at maturity between the sexes, with the later maturing sex achieving larger body sizes (Kozlowski, 1989). In fact, there are many examples of males that mature earlier than females in snakes (Parker and Plummer, 1987). Knowledge of individual age is crucial to our understanding of sexually dimorphic growth and its impact on the behavior and ecology of animals.

Through the use of individuals born in the laboratory, we have further identified the role of gonadal hormones in mediating differential growth in this species. Our results indicate that there is a dimorphic nature to the effects of the major sex steroids E_2 and T in males and females. Blocking the endogenous effects of E_2 had negative effects on

female growth, but not on male growth. Blocking the effects of endogenous E_2 or T in males had no effect on growth indicating that the presence of either of these hormones is sufficient for male-like growth.

While we have found differences with regard to the effect of gonadal hormones, their influence on sexual size dimorphism are not necessarily adaptive and may be incidental (Lande, 1980). Sexual size dimorphism may be influenced by completely separate factors with respect to males and females. In fact, sexual dimorphism could be the product of non-adaptive factors as reviewed by Fairbairn (1990).

Understanding the factors influencing sexual dimorphism in animals is crucial to gaining a full comprehension of the forces affecting ecological and selective pressures on the biology and evolution of a species. The factors that affect sexual dimorphism may occur during embryonic development, during adolescence, at maturity or in adulthood. Identifying the factors that mediate variability in growth and development will increase our understanding of the interrelationships between adult sexual dimorphism associated with size and its impacts on life history and ecology. Differential growth of the sexes can reflect adaptations to environmental situations, thereby maintaining mating and reproductive success, which ultimately results in species survivorship.

Future Considerations

While our research indicates that some extent of sexual dimorphism exists at birth in this species, we suggest that this condition is a result of differential maternal allocation of energy. Pre-natal investigation and manipulation of the factors contributing to the

numbers and ratio of individuals born would be informative. This could prove difficult in a live bearing snake because high levels of stress associated with manipulations during gestation may inhibit the production of offspring (personal observation). However, manipulations regarding stressful events during gestation may negatively influence the size and number of individuals produced. Investigations of the effects of stress and the allocation of parental investment would be informative. To date the effects of the removal of the neonatal ovary on growth are unknown. Experimentation in this respect would help clarify our findings as well as those of Crews *et al.*, (1985). In addition, other gonadal hormones or metabolites may have an influential role in differential growth of garter snakes. Finally, we have shown that neonatal growth from birth until a period of time coinciding with winter dormancy is unaffected by the major sex steroids (this study) or specific food and temperature regimes (Lerner and Mason in prep.). It would appear that growth during this period is determined previous to birth. Investigation of this phenomenon would be extremely valuable.

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