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**Factors affecting variation in litter sex ratios and offspring
size in eastern garter snakes, *Thamnophis sirtalis sirtalis*, and
northern ribbon snakes, *Thamnophis sauritus septentrionalis***

by Sophie Jane Sommerer

A thesis submitted to
the Faculty of Graduate Studies and Research
in partial fulfilment of the
requirements for the degree of
Master of Science

Department of Biology

Carleton University

Ottawa, Ontario

May 2000

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**Factors affecting variation in litter sex ratios and offspring
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in partial fulfilment of the requirements of
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Chair

Supervisor

2 August 2000
Date

ABSTRACT

I investigated the influence of females on their young in eastern garter snakes, *Thamnophis sirtalis sirtalis*, and northern ribbon snakes, *Thamnophis sauritus septentrionalis*. In Chapter 1, I found that sex ratios varied greatly among litters. In ribbon snakes, the proportion of males increased with neonate size, but not with maternal size. Sex ratios varied independently of maternal size in garter snakes. The unexpectedly high number of stillborn neonates was not due to differences in maternal size, litter size, or time in captivity. In Chapter 2, I examined offspring size variation in both species. Using a path analysis, I found that larger litters were born to larger females earlier in the season. Although offspring size increased with maternal size, I also observed a trade-off between offspring size and number. Though this study expanded our understanding of maternal effects in snakes, the general patterns of maternal control over offspring characteristics remain unclear.

ACKNOWLEDGEMENTS

It is hard to believe that two years have passed so quickly. Now that I have finally finished this thesis, I am left with so many people to thank...

First, I would like to thank my supervisor, Dr. Pat Weatherhead, for taking me on even though I had never touched a snake before I started. I owe a great deal to him for his guidance and support, and most importantly, for reading and commenting on the many drafts of this work. I would also like to thank my committee members, Drs. Katie Gilmour and Vance Trudeau, for their helpful suggestions along the way.

I thank Queen's University for the use of their wonderful Biological Station. I especially appreciate the assistance of the staff at QUBS, and of the many researchers who helped in my snake-catching efforts (even if some of them were only doing it to save their nests from predation). Although the field work was exhausting, the company was most enjoyable.

For helping me with all of my field work, I must thank Kelley Kissner, Jen Gilchrist, Pat Begin, Stephanie Doucet, and Greg Brown for assisting in data collection, as well as the black rat snake team (Gabriel Blouin-Demers, Heather McCracken, Tony Volk, and Alissa Moenting) for occupying the "other side of the lab".

At Carleton, I was welcomed by our neighbours in the Forbes lab. Thank you to Brian Leung, Dean McCurdy, Chris Yourth, Kerry Hecker, Dave McCruer and Mark Forbes. I have enjoyed our many conversations about science, statistics, and everything in between. A huge thank you also to Darren Bender, our resident computer expert,

especially for helping me set up Internet access on my own computer in the lab. (It has been a welcome distraction in the final stages of writing.)

My own lab-mates have been so helpful both in the field and in the lab. Thank you to Tony Volk (whose optimism is inspiring), Heather McCracken (who has always been able to put a smile on everybody's face), and Gabriel Blouin-Demers (who let me help with snake surgeries even though my enthusiasm was sickening). Lastly, I owe a huge debt to Kelley Kissner. She accepted me as her field assistant without having met me and taught me almost everything I know about working with snakes. I definitely would not have made it this far without her.

Last, but not least, I extend many thanks to my family and friends, especially my boyfriend, Cameron Chabot. They have all been so supportive, even though they couldn't understand why anyone would even want to hold a snake, let alone study them. Without them, I would never have made it through two university degrees and emerged smiling.

Thank you all so much!

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GENERAL INTRODUCTION

A female's investment of materials and energy in reproduction is necessarily limited by the amount that she invests in other physiological processes like growth (Stearns 1992). Selection acts on a female so that she allocates her resources in a way that maximises the number of her offspring that survive to reproduce (Trivers 1974). Therefore, the magnitude of her reproductive effort in a given season might influence various characteristics of her litter, including the number of offspring that she produces (Roff 1992), the size and condition of her offspring (Clutton-Brock 1991), and/or the sex ratio of her litter (Trivers and Willard 1973). In addition to being determined by genes and the interactions of genes with the environment, offspring phenotypes might depend on maternal effects, which are defined as any direct effect of a parent's phenotype on the phenotype of its offspring (Bernardo 1996). Thus, females could have direct control over the quality of their young beyond their genetic contribution.

Many aspects of reproductive biology are poorly understood in snakes compared to other reptilian groups (Seigel and Ford 1987). For example, very little information exists on the factors that influence offspring fitness (Parker and Plummer 1987). Because most snakes provide no parental care to their offspring, they lend themselves well to the study of the effects of mothers on their offspring. With parental investment ending at the time of parturition, maternal influences are limited to those that occur before birth. Therefore, measurements of young at birth can be used as indices of offspring quality. In my thesis, I have investigated the effects that a mother has on the number, size and sex of

her young in two live-bearing snake species, eastern garter snakes (*Thamnophis sirtalis sirtalis*) and northern ribbon snakes (*Thamnophis sauritus septentrionalis*).

In Chapter 1, I investigated whether females adjusted the sex ratio of their litters adaptively. Although many studies have supported Fisher's (1958) theory that parents should be selected to produce equal numbers of sons and daughters, more recent work has shown that many females can and do adjust their litter sex ratios in response to their current condition (Trivers and Willard 1973). However, evidence for this pattern of adaptive sex allocation in snakes is somewhat contradictory (Lemen and Voris 1981, Dunlap and Lang 1990, Plummer 1992, Weatherhead et al. 1998). By reinvestigating one study species with direct measurement of offspring size (*T. sirtalis*) and by adding another closely-related species to the analysis (*T. sauritus*), I have established whether the large amount of variation observed in litter sex ratios can be explained by a female's attempt to adaptively allocate the sex of her young.

In Chapter 2, I focused on the variation in offspring size within and among litters. In particular, I examined the validity of the optimal offspring size theory, given that offspring size varies significantly within populations across many taxa (Roff 1992, Stearns 1992, Bernardo 1996a). In previous studies of snakes, offspring size has been shown to be related to maternal size (Ford and Killebrew 1983, Ford and Karges 1987, King 1993), offspring number (King 1993, Madsen and Shine 1996, Weatherhead et al. 1999), and date of parturition (King 1993). Using univariate and multivariate tests, I investigated the contribution of maternal characteristics (snout-to-vent length, or SVL, and mass), litter

characteristics (number of offspring), and environmental characteristics (date of parturition, time in captivity) to offspring size variation in garter and ribbon snakes.

CHAPTER 1:

Offspring sex ratio variation in eastern garter snakes, *Thamnophis sirtalis sirtalis*, and northern ribbon snakes, *Thamnophis sauritus septentrionalis*

Introduction

Mendelian sex determination predicts a one-to-one ratio of males to females at birth in any sexually reproducing population (Spieth 1974), and indeed, in many species, primary sex ratios do not differ significantly from equality (Charnov 1982, Parker and Plummer 1987). However, from a theoretical perspective, there should be a substantial selective advantage to females that control the sex of their progeny (Fisher 1958, Charnov 1982). Viewed this way, equal sex ratios become the outcome of adaptive sex allocation decisions by females, rather than the non-adaptive outcome of random segregation of sex chromosomes. More interesting, however, is the expectation that adaptive sex allocation should also produce sex ratios that deviate significantly from equality under particular circumstances. The goal of my study was to examine sex ratio variation in two species of snakes to determine whether sex ratios vary non-randomly, as predicted from adaptive sex allocation theory.

The first hypothesis for adaptive sex allocation was presented by Fisher (1958), who argued that natural selection would favour parents that invest equally in their sons and daughters because the reproductive value of males and females in sexually reproducing species is equal. Because the best reproductive strategy for each individual must be a compromise between conflicting demands for production of the largest possible

number of offspring and production of offspring of the highest possible individual fitness (Fisher 1958, Willson and Pianka 1963), an imbalance in the litter sex ratio may evolve if the production of one sex requires greater parental expenditure of energy than the production of the other sex (Maynard Smith 1980). It is only when sons require the same parental investment as daughters that the expected population sex ratio is one-to-one. Despite the antiquity of Fisher's sex ratio theory, its empirical support remains ambiguous. For example, female-biased fledgling sex ratios in birds in which females are smaller than males have been interpreted as supporting the theory because overproducing less expensive females serves to balance overall parental investment in sons and daughters. However, Weatherhead and Teather (1991) showed that these biases are also expected as a consequence of the larger male nestlings' higher likelihood of starving when food is scarce, and suggested that the available data are more consistent with this non-adaptive explanation for female-biased sex ratios.

A second theory on adaptive sex allocation arose with the observation that many insect and mite populations with high levels of brother-sister mating have highly female-biased sex ratios (Hamilton 1967). This result was explained by Hamilton's (1967) theory of local mate competition which states that if one sex suffers a greater reduction in fitness through competition with siblings of the same sex, then the mother is selected to bias the sex ratio towards the sex that suffers less competition. Patterns of sex allocation observed in numerous studies of parasitoid wasps and fig wasps are consistent with predictions based on local mate competition theory (Godfray and Werren 1996).

The third general theory for adaptive sex allocation, and that which is most relevant to my study, was proposed by Trivers and Willard (1973). They suggested that, under certain well-defined conditions, natural selection favours systematic deviations from a one-to-one sex ratio at conception within litters. Total parental investment of an individual in a given reproductive season is the sum of its input into each of its offspring produced during that season (Trivers 1972). Although the sex ratio of a litter could be biased towards the smaller sex to allow the production of a maximum number of offspring (Parker and Plummer 1987), it is possible that those females who can invest more in their offspring may favour the overproduction of the sex that grows larger (Shine 1978). Indeed, individual females produce litters or clutches with sex ratios varying from highly male-biased to highly female-biased, although the overall sex ratio does not differ from 0.50 at the population level (Bull 1983). Godfray and Werren (1996) cite numerous studies that have shown that sex ratios vary as predicted by Trivers and Willard's (1973) sex allocation theory. For instance, sex ratio is correlated with host size in many parasitoids, where ovipositing mothers lay eggs of the sex that will benefit most (in terms of fitness) from developing in a host of a given size. In many mammals that have dominance hierarchies, sex ratios also vary among litters such that a female produces more offspring of the sex that benefits most, or suffers least, from her current condition or rank (Godfray and Werren 1996). For example, Silk (1983) showed that females of several species of primates (*Macaca radiata*, *M. mulatta*, *Papio cynocephalus*) adaptively adjusted the sex ratio of their progeny in relation to their dominance status, even though the population sex ratio did not differ from one-to-one. In all three species, low-ranking

females produced more male offspring than female offspring, and high-ranking females produced either disproportionately female offspring (in two species) or equal numbers of male and female offspring (in one species). In these mammals, sons dispersed while daughters remained with their natal group and thus, daughters acquired their social status from their mothers. Because the daughters of low-ranking females were destined to become low-ranking, with consequent low fitness, low-ranking females benefited from producing mostly sons. Conversely, high-ranking females benefited from producing predominantly daughters (Silk 1983).

As outlined above, a substantial amount of information has been accumulated on population and litter sex ratios in various organisms. However, reptiles are not as well represented in studies of sex ratios, except for those that address the phenomenon of environmental sex determination (Bull 1983). Although little is known about sex ratios in snakes, they are excellent candidates for such investigations. First, because snakes provide no parental care to their offspring after parturition (Seigel and Ford 1987, Clutton-Brock 1991), many complicating factors present in studies of other organisms (like competition between siblings or feeding rates after birth) are eliminated. Thus, a female's influence on her young is limited to factors that she can control before and during gestation. One way that she might affect the fitness of her offspring would be to manipulate the sex of her offspring and, thus, the ratio in which males and females are produced. Second, many snake species are sexually dimorphic in size (Shine 1994), suggesting that one sex might benefit more from large size at birth. Thus, there is potential for a female to manipulate the sex ratio of her litter in response to her current condition. Third, in some populations of

snakes, dispersal is limited to members of one sex. In those species, members of the sex that does not disperse would suffer greater degrees of local mate competition (Hamilton 1967), and females might attempt to overproduce the sex that disperses.

Studies of snake sex ratios have provided some evidence relevant to each of the theories of adaptive sex allocation. As predicted by Fisher's (1958) theory of sex allocation, early studies of snake sex ratios showed that sons and daughters were produced in equal numbers (Forbes 1940). More recently, Shine and Bull (1977) reviewed offspring sex ratios of 21 viviparous snake species and found a definite male-bias in only one species, *Notechis scutatus*. In northern water snakes, *Nerodia sipedon*, neither the overall population sex ratio nor the sex ratio of neonates differed significantly from one-to-one (Weatherhead et al. 1995). The overall natal sex ratios did not differ significantly from 0.50 in the marine snake, *Enhydrina schistosa* (Lemen and Voris 1981), or in the common garter snake, *Thamnophis sirtalis*, the ribbon snake, *T. sauritus*, or Butler's garter snake, *T. butleri* (Carpenter 1952). In another population of *T. sirtalis*, neither the mean litter sex ratio nor the overall sex ratio of neonates differed from 0.50 (Dunlap and Lang 1990).

Although population sex ratios do not differ significantly from one-to-one in most snake species, there is evidence that snakes do allocate offspring sex adaptively as predicted by Hamilton's (1967) local mate competition theory. Madsen and Shine (1992) showed that, in adders (*Vipera berus*), where brothers compete for matings, females produced more daughters than sons. Overall, females outnumbered males in adder populations, as expected for a species in which local mate competition is more severe

among males than among females. There is also evidence that snakes might adjust the sex ratio of their offspring in relation to competition for resources other than mates. For example, Gutzke and colleagues (1985) showed that overall sex ratios of adult and hatchling bullsnakes (*Pituophis melanoleucus*) were both male-biased (0.672 and 0.667, respectively). They reasoned that this bias resulted from more severe local resource competition among females, who remained close to their mothers, than among males, who dispersed from their natal areas. However, they failed to show that local resource competition existed in this species. Another problem with their study was that they only considered the differences in competition with genetically-related individuals in and away from the natal area. To demonstrate that bullsnakes would benefit from a skewed sex ratio, Gutzke et al. (1985) needed to show that the density of snakes was higher in the natal area, thus showing that competition with all (unrelated and related) individuals was lower for those individuals that dispersed.

To establish whether adaptive sex allocation occurs in a population whose overall sex ratio does not differ from equality, it is necessary to determine whether the variation of litter sex ratios agrees with the predictions of Trivers and Willard's (1973) theory. Only four known studies have tested this theory in snakes by comparing litter sex ratios to factors such as maternal size, neonate size and date of birth. In one of the studies, litter sex ratios varied independently of maternal size in *Nerodia rhombifer* (Plummer 1992). In the remaining three studies, litter sex ratios were shown to be related to maternal size and, in one of these species, also to offspring size. Lemen and Voris (1981) studied the marine snake *E. schistosa* and found that larger females produced proportionately more female

offspring. They suggested that this relationship existed because neonate and maternal size were positively related and because daughters were larger than sons. However, because they did not measure offspring size, they could not test the validity of either of these assumptions. In garter snakes, *T. sirtalis*, litter sex ratios varied from 0.23 to 0.80, with the proportion of males increasing as maternal size increased (Dunlap and Lang 1990). In their study, Dunlap and Lang (1990) also did not measure offspring size directly, but cited studies of other populations that strongly suggested that male neonates were larger than female neonates within a litter. Consequently, they reasoned that small females produced smaller, predominantly female young, and large females produced larger, predominantly male young. However, there were two potential problems with their conclusion. First, their assumption that males were larger than females within a litter was likely erroneous. Of the three studies of *Thamnophis* species that were cited, two of them tested for and failed to find significant differences between male and female offspring size (Gregory 1977, Ford and Killebrew 1983), and the third did not even consider male and female neonates separately (Ford and Karges 1987). Furthermore, at least one other study of *T. sirtalis* measuring sexual size dimorphism at birth found that offspring size did not differ between male and female neonates (Larsen et al. 1993). Second, the pattern of sex ratio variation described by Dunlap and Lang (1990) is opposite to that predicted if females adaptively select the sex of their offspring. Because sexual size dimorphism is female-biased in garter snakes (Shine 1994), large size should provide a greater benefit to females than to males. The problems with Dunlap and Lang's (1990) study justify the need for a study that measures sex ratio and neonate size concurrently within litters in garter snakes.

Such a study was conducted in northern water snakes, *N. sipedon*, where litters with larger offspring were found to be female-biased as expected, because female neonates should benefit more from larger size at birth (Weatherhead et al. 1998). In that study, the proportion of male young in a litter also decreased with maternal size, although maternal size and neonate size were not correlated. Thus, Weatherhead et al. (1998) suggested that the negative relationships between sex ratio and maternal or neonate size were independent of each other.

Another factor with which sex ratio has been proposed to vary non-randomly is date of parturition, because earlier birth in a seasonal environment provides neonates with greater opportunity for growth before they enter hibernation. Such a result was suggested by Dunlap and Lang (1990) upon reanalysis of the sex ratio data from Osgood's (1978) study of common water snakes, *Nerodia fasciata*. In Osgood's (1978) study, snakes maintained at warmer temperatures produced more daughters than those maintained at cooler temperatures. Because parturition is accelerated at warmer gestation temperatures (Brown and Weatherhead 1997), females held at warmer temperatures would have likely given birth to their female-biased litters earlier than those females held at cooler temperatures gave birth to their male-biased litters. Because size dimorphism is female-biased in this species, female neonates should benefit more than males from additional time for growth prior to hibernation. If timing of parturition depended only on the timing of ovulation, females could bias the sex of their litter accordingly, producing more female offspring if they ovulated early and more male offspring if they ovulated late. However, date of parturition also depends on the duration of gestation, which was manipulated in

Osgood's (1978) study by holding females at different temperatures. Therefore, the relationship between sex ratio and date of birth does not seem to be adaptive in *N. fasciata*. Instead, it is possibly the result of differential mortality of male and female embryos. In a more recent study of *N. sipedon*, where females were held in different treatments during gestation, sex ratio and date of parturition were not related (Weatherhead et al. 1998). This result was not surprising because females could not predict the duration of gestation at the time of ovulation, when sex was determined. Furthermore, Weatherhead et al. (1998) found additional evidence for differential mortality in their study. Stillborn neonates were predominantly male suggesting that male embryos might be more vulnerable to high temperatures during gestation.

As outlined previously, four studies of litter sex ratios in snakes have generated conflicting evidence of Trivers and Willard's (1973) adaptive sex allocation theory, leaving many questions unanswered. First, it has not been established that maternal and neonate size are correlated in garter snakes, nor whether they influence sex ratio variation together or independently of one another. Second, the previous study of garter snakes assumed that the observed sex ratio variation occurred because of male-biased sexual size dimorphism of neonates, although this assumption was based on a misinterpretation of existing evidence in *Thamnophis* species. Third, sex ratios have not been shown to vary consistently across species of snakes. In fact, the predicted relationship between sex ratio and neonate size was shown in only one of the four studies carried out to date. My study of sex ratio variation in garter and ribbon snakes addresses many of these unresolved problems. First, I gathered data on adult male and female snakes to verify the existence of

female-biased dimorphism in garter and ribbon snakes. Furthermore, I determined whether this female-biased size dimorphism was evident at birth as it was in *Nerodia sipedon* (Weatherhead et al. 1995) or whether, as in other populations of *T. sirtalis*, male neonate size was equal to that of females (Gregory 1977, Larsen et al. 1993). I also determined whether maternal and neonate size were correlated and whether they influenced sex ratio variation together or independently of one another. Additionally, I investigated the same questions in two species of snakes, thus adding another population of garter snakes and another species to the available evidence regarding sex ratio variation in snakes. Ribbon and garter snakes are members of the same genus and coexist in many locations. I studied both species at the same time, so environmental and procedural differences were eliminated. Similar findings in garter and ribbon snakes would strengthen the conclusions of my study. Lastly, I have compared the number of male and female young that are stillborn to determine whether male embryos are more susceptible to mortality during development, as they were in *N. sipedon* (Weatherhead et al. 1998).

The overall objectives of my study were: (1) to determine whether the variation of litter sex ratios was non-random, that is, whether sex ratios varied predictably with any maternal, neonate or litter trait; and (2) to determine whether any non-random variation that existed was consistent with adaptive sex allocation theory. If garter and ribbon snake females adaptively adjusted the sex ratios of their litters, I expected that litters with larger offspring would be predominantly female, while male-biased litters consisted of smaller offspring. If maternal and neonate size were correlated, I also predicted that larger females would give birth to larger offspring that were predominantly female, and smaller females

would give birth to male-biased litters of smaller offspring. Lastly, because the females in my study experienced similar conditions during gestation, variation in date of parturition depended primarily on variation in date of ovulation. Therefore, if females adjust the sex ratio of their litters according to their timing of ovulation, litters born earlier should be biased toward females.

Although my research does not directly address the mechanism by which a female might control the sex ratio of her offspring, the means must exist if she is to do so. Because the sex of snakes is determined chromosomally (Bull 1980), temperature will have no direct effect on the overall sex ratio of a litter, thus precluding females from adjusting the sex ratios of their offspring by behavioural thermoregulation. Furthermore, because females are the heterogametic sex (Bull 1980), intrauterine discrimination among sperm can not be used to control offspring sex, because sperm are not the gametes that determine sex. Therefore, adaptive sex ratio variation must result from a maternal influence, which could take one of two forms. First, a female might possess a mechanism that allows her to detect the sex of an ovum at or before fertilisation, providing the potential to selectively reabsorb ova of the less desirable sex. However, evidence for egg resorption in squamate reptiles is weak to non-existent (Blackburn 1998). Alternatively, non-random segregation of sex chromosomes into ova might occur, allowing a female to bias the sex of her offspring before fertilisation. In both cases, the maternal strategies of adaptive sex allocation involve mechanisms that operate prior to the gestation period.

Materials and Methods

I conducted this study from April to September of 1997 to 1999 at the Queen's University Biological Station (QUBS) in eastern Ontario, Canada (45° 37' N, 76° 13' W). During the study, I captured garter and ribbon snakes by hand from forests, marshes, fields and roadways on QUBS property. I relied on opportunistic sightings to find snakes (that were involved in numerous activities, including feeding, basking and moving), and attempted to capture every snake that was seen. Other researchers at QUBS also caught snakes they saw, and brought them into captivity. In late April 1999, I captured additional snakes that emerged from a hibernation site of garter snakes, located approximately 10 km from QUBS property. In the lab, I recorded total length, tail length, snout-to-vent length (SVL), and mass of each snake within 12 hours of capture. I measured total length by running a string along the body of the snake, following all of the contours, and then measuring the string. I used the mean of two replicate measurements to represent total length of each snake. When the replicates differed by more than 1 cm, I remeasured the snake, and calculated the mean of the measurements that differed by less than 1 cm. I measured tail length by placing the tail directly on the ruler, and calculated SVL as the difference between mean total length and tail length. I determined the sex of each snake by probing the cloaca for the presence of hemipenes. I marked adult male snakes with a unique brand on the tail scales, to facilitate individual recognition upon recapture, then released them at the point of capture. I implanted a transponder under the skin of adult female snakes to ensure accurate identification over the course of the study. The transponder was injected under the skin of the snake, and the small wound was sealed with

surgical glue. The use of transponders to mark snakes is widely accepted as safe and effective.

During the study, I housed snakes in outdoor enclosures from the time of capture until expected parturition. I held garter snakes in outdoor enclosures constructed of painted sheet metal (approximately 1m high) that measured approximately 5 m in diameter. I allowed natural vegetation to grow within each enclosure and provided the snakes with wood and leaf litter, as well as a small cement structure, for shelter. I placed a large water dish in each enclosure for drinking and submersion. I held ribbon snakes in similar enclosures, larger in size (5m x 10m) with a larger water body for swimming. Again, I let natural vegetation grow in the enclosures, and provided wood for additional shelter. All enclosures were surrounded by electric fencing to discourage terrestrial predators from entering, and birds were deterred by flagging tape strung above the enclosures.

Females were held in enclosures for the majority of the study. At the time of expected parturition, I transferred them to individual cages in a heated room. I allowed the temperature of the rooms to fluctuate with environmental conditions, but maintained it above 20°C. I held some snakes in plastic aquaria (ca. 48 x 48 x 48 cm) lined with artificial grass matting and containing heating rocks for thermoregulation. In 1998, I placed some snakes in plastic boxes (ca. 33 x 20 x 9 cm) without external heat sources, but the temperature of the room was held above 22°C. In 1999, I provided snakes in plastic boxes with intermittent access to heat from incandescent light bulbs. Because the light bulbs shone on one end of their boxes, the snakes could thermoregulate by selectively

positioning themselves close to or away from the heat source. Although there were some differences in holding conditions among females in captivity, I assumed that the differences did not have dramatic effects on the females because they occurred only for a few weeks in late gestation. In enclosures and in captivity, I provided water to all snakes *ad libitum*. I fed the snakes with minnows (both live and previously frozen) at least twice per week. I remeasured all females when I transferred them from enclosures to captivity, then weekly until parturition. Once a female gave birth or once I determined that she was not gravid, I released her at the point of capture.

At the time of parturition, I collected all neonates and follicles for measurement and recorded date of parturition as Julian date. I measured the SVL and mass of each adult female within 24 hours of parturition and recorded the number of neonates (live and stillborn) in her litter. I measured SVL, tail length, mass and sex for each neonate (live and stillborn) within 24 hours of parturition. I determined neonate SVL and tail length (to the nearest 1 mm) by placing the neonate directly on a ruler, and measured mass to the nearest 0.1 g. I determined the sex of each neonate by probing the cloaca for the presence of hemipenes. Because the validity of my findings relied greatly upon the accurate identification of neonate sex, in 1998 and 1999 I determined the sex of all neonates using a second method, blind to the initial identification of sex. This involved applying a gentle pressure in the tail towards the cloaca to evert the hemipenes, if present. In only one of the 101 litters that were checked, I had to reassign the sex of one neonate.

Statistical analyses

I represented the sex ratio of a litter by the proportion of male neonates it contained. When the data did not meet the assumptions of parametric tests, I transformed measurements of maternal size and time in captivity with log-transformations, and litter size with square-root transformations to be used in linear models. I used non-parametric tests of the untransformed data when the assumptions were not met. Because the size measurements of neonates within a litter were not normally distributed, I used median values of SVL, tail length and mass to represent a litter's neonate measurements.

In all of my analyses, I used SVL of a female after parturition to represent maternal SVL, whereas previous studies used SVL at the time of capture (Dunlap and Lang 1990, Weatherhead et al. 1998). In my study, where females were captured over several months, I decided that it was more appropriate to use SVL after parturition to reduce variation in measurements due to timing of capture. I did verify that, although SVL increased from time of capture to parturition, both measures of SVL were highly correlated in garter snakes (linear regression, $F_{1,66}=213.7$, $p<0.0001$, $r^2=0.76$) and ribbon snakes (linear regression, $F_{1,40}=227.3$, $p<0.0001$, $r^2=0.85$).

I used analyses of covariance (ANCOVA) to determine whether sex ratio varied with maternal, neonate or litter traits while controlling for the year of study. In these analyses, litter sex ratio was the dependent variable, the maternal, neonate or litter trait was the continuous independent variable, and year was the categorical independent variable. I included an interaction term between the independent variables, but removed it and reported only the reduced model results when the interaction term did not contribute to the model with a significance level of $p<0.10$. I conducted all analyses separately for

garter and ribbon snakes using JMP (version 3.1) statistical software (SAS Institute Inc. 1994), and accepted significance at 0.05 level.

Results

During this study, I gathered data on a total of 70 litters of garter snakes (Table 1-1) and 42 litters of ribbon snakes (Table 1-2). Overall, 441 female and 445 male garter snake neonates were born in captivity, corresponding to a natal sex ratio of 0.502. Litter sex ratios ranged from 0.09 to 1.00 in garter snakes, with a mean of 0.512, which did not differ significantly from equality (t-test, $t=0.60$, $p=0.55$, $n=70$). Of the 382 ribbon snakes born in captivity during my study, 196 were female and 186 were male (a sex ratio of 0.480). In ribbon snakes, litter sex ratios ranged from 0.10 to 0.90, with a mean of 0.495, which did not differ significantly from 0.50 (t-test, $t=-0.19$, $p=0.85$, $n=42$).

There was some variation in maternal, neonate, and litter characteristics among the years of study in garter and ribbon snakes (Table 1-1, Table 1-2, respectively). In garter snakes, maternal SVL was significantly longer in 1997 than in 1999 (ANOVA, $F_{2,67}=5.43$, $p=0.007$, $r^2=0.14$; Tukey test, $p<0.05$), and post-partum mass seemed to differ somewhat among years (ANOVA, $F_{2,67}=2.91$, $p=0.06$, $r^2=0.08$). Neither neonate size (SVL or mass) nor litter size differed significantly among years in garter snakes (ANOVA, $p>0.16$). In ribbon snakes, none of the measurements of maternal size, neonate size or litter size differed significantly among years (ANOVA, $p>0.23$). Date of parturition differed significantly among years in both study species. In garter snakes, birth date was significantly later in 1997 and 1999 than in 1998 (ANOVA, $F_{2,67}=12.55$, $p<0.0001$,

Table 1-1. Characteristics of litters of eastern garter snakes, *Thamnophis sirtalis*.Significant differences among years are indicated (* $p < 0.01$; *** $p < 0.0001$).

	1997	1998	1999	Overall
# of litters	4	32	34	70
Litter size				
Mean	17.3	12.9	12.1	12.8
Range	12-25	4-28	5-20	4-28
Sex ratio				
Mean	0.378	0.552	0.491	0.512
Range	0.24-0.56	0.30-0.79	0.09-1.00	0.09-1.00
Birth date				
Mean	224.0***	207.0***	214.4***	211.6
Range	215-235	191-223	204-237	191-237
Relative birth date				
Mean	0	0	0.041	0.020
Range	(-9)-11	(-16)-16	(-10.4)-22.6	(-16)-22.6
Maternal SVL (cm)				
Mean	58.50*	54.06	51.49*	53.07
Range	53.4-66.2	47.0-64.7	43.9-60.4	43.9-66.2
Maternal mass (g)				
Mean	85.8	70.6	62.4	67.5
Range	54-137	45-114	42-96	42-137
Median neonate SVL (cm)				
Mean	14.83	14.71	14.40	14.57
Range	14.0-15.3	13.2-16.3	12.7-16.2	12.7-16.3
Median neonate mass (g)				
Mean	1.83	1.74	1.62	1.69
Range	1.4-2.4	1.2-2.4	1.0-2.3	1.0-2.4
# stillborn				
Mean	0	4.97	2.97	3.71
Range	0	0-19	0-12	0-19

Table 1-2. Characteristics of litters of northern ribbon snakes, *Thamnophis sauritus*.Significant differences among years are indicated (***) $p < 0.0001$.

	1997	1998	1999	Overall
# of litters	7	9	26	42
Litter size				
Mean	9.3	8.9	8.9	9.0
Range	4-12	2-21	3-14	2-21
Sex ratio				
Mean	0.596	0.422	0.492	0.495
Range	0.36-0.80	0.10-0.71	0.10-0.90	0.10-0.90
Birth date				
Mean	223.9***	202.7***	216.3***	214.6
Range	218-235	194-213	202-226	194-235
Relative birth date				
Mean	0	0	0	0
Range	(-5.9)-11.1	(-8.7)-10.3	(-14.3)-9.7	(-14.3)-11.1
Maternal SVL (cm)				
Mean	50.26	50.34	49.27	49.66
Range	46.9-54.8	44.6-59.7	43.2-57.9	43.2-59.7
Maternal mass (g)				
Mean	43.4	46.7	44.2	44.6
Range	31-63	36-65	27-69	27-69
Median neonate SVL (cm)				
Mean	15.15	14.93	14.92	14.96
Range	14.6-15.7	14.2-15.7	13.6-17.0	13.6-17.0
Median neonate mass (g)				
Mean	1.52	1.33	1.43	1.42
Range	1.3-1.8	1.1-1.5	1.0-2.0	1.0-2.0
# stillborn				
Mean	1.57	1.78	1.12	1.33
Range	0-5	0-6	0-10	0-10

$r^2=0.27$; Tukey test, $p<0.05$). Date of birth in ribbon snakes was also significantly earlier in 1998 than in 1997 and 1999, as well as being significantly earlier in 1999 than in 1997 (ANOVA, $F_{2,39}=27.89$, $p<0.0001$, $r^2=0.59$; Tukey test, $p<0.05$). Because date of parturition differed among years, I have conducted the remaining analyses, controlling for year effects, first with absolute date of parturition and then with relative date of parturition (defined as the number of days before or after the mean date of parturition for that species in that season). The results from the second set of analyses are only presented when they differ qualitatively from the first.

Accuracy of sexing technique

In adult and neonate garter snakes, males' tails are proportionately longer than females' tails (Fitch 1965). I compared the lengths of male and female tails to confirm that this pattern existed in my study species. I considered tail length as a proportion of a snake's SVL to control for differences in body size. In adult garter snakes (GS) and ribbon snakes (RS), tails were significantly longer in males than females, relative to their SVL (GS: Mann-Whitney test, $S=14426$, $p<0.0001$, $n=208$, 145; RS: Mann-Whitney test, $S=4047$, $p<0.0001$, $n=53$, 62).

To assess the accuracy of my assignment of sex in neonates, I also compared the length of male and female neonates' tails. Among all garter snake neonates, relative tail length was significantly greater in males than in females (Mann-Whitney test, $S=195807.5$, $p<0.0001$, $n=368$, 382). Within litters, relative tail length was significantly greater in males than in females (paired t-test, $t=19.68$, $p<0.0001$, $n=61$). Relative tail length was significantly greater in males than in females for all ribbon snake neonates (Mann-Whitney

test, $S=42630.5$, $p<0.0001$, $n=196$, 186) and within ribbon snake litters (paired t-test, $t=5.43$, $p<0.0001$, $n=40$). When the length of a neonate's tail indicated that its sex may have been assigned incorrectly by the probing of hemipenes, I verified its sex by everting the hemipenes. In all instances, I found that the initial identification of sex was correct, and concluded that the individual had an unusually long or short tail for its sex.

Occurrence of stillborn young

An unexpectedly large number of females gave birth to stillborn young during my study. In total, 35 of 70 garter snake litters and 18 of 42 ribbon snake litters contained stillborn young. Because there was such a high incidence of stillbirths in my study, I attempted to determine what factors contributed to the presence or absence of stillborn young in a litter. The presence of stillborn young might depend on the size of the mother because a smaller female might not be able to provide enough resources to all of her offspring to ensure their survival. An increase in litter size might also increase the possibility that a litter will contain stillborn young because a female who has committed to producing a large number of offspring might sacrifice one to ensure the survival of the others. Overall, I found that neither the presence nor the number of stillborn young depended on maternal size or litter size in garter or ribbon snakes (Table 1-3). The probability that a garter snake gave birth to stillborn young tended to decrease as maternal mass increased and to increase as litter size increased, although neither relationship was significant.

It is also possible that an increase in the length of time that a female spent in captivity might have increased the chance that her litter would contain stillborn young,

Table 1-3. Effects of maternal size (SVL and mass) and litter size on the presence and number of stillborn young in garter and ribbon snake litters. Presence of stillborn young was analysed in a multiple logistic regression against the independent variables. Number of stillborn young (in those litters containing stillborn young) was analysed in a multiple linear regression against the independent variables.

Species	Variable	Presence of stillborn young				Number of stillborn young			
		χ^2	p	R ²	n	F	df	p	R ²
Garter Snakes	Overall analysis	3.43	0.33	0.04	70	2.22	3,31	0.11	0.18
	Maternal SVL	1.04	0.31			1.94		0.17	
	Maternal mass	1.33	0.25			3.05		0.09	
	Litter size	1.57	0.21			3.08		0.09	
Ribbon Snakes	Overall analysis	3.13	0.37	0.05	42	1.19	3,14	0.35	0.20
	Maternal SVL	0.32	0.57			2.23		0.16	
	Maternal mass	0.96	0.33			2.66		0.13	
	Litter size	1.52	0.22			1.27		0.28	

because captive conditions are known to have a negative effect on reproductive factors in snakes (Farr and Gregory 1991). In my study, litters with stillborn young tended to be born later than those without stillborn young, so I included date of birth and year in this analysis also. I found no evidence that an increase in the length of time that a female was held in captivity contributed to the high occurrence of stillborn young in either garter or ribbon snakes (Table 1-4). In fact, the number of stillborn young tended to decrease with time in captivity in garter snake litters containing stillborn young. However, the probability that a litter contained stillborn young increased as date of birth increased in both garter and ribbon snakes, and the number of stillborn young in garter snake litters that contained stillborn young tended to increase with date of birth and year of study. When I conducted these analyses with relative date of birth, year no longer contributed to any of the differences in probability or number of stillborn young in either study species.

Although the probability that a female gave birth to stillborn young was not related to the length of time she spent in captivity, the conditions in captivity may have contributed to the occurrence of stillbirths during my study. For example, the importance of thermoregulation to gravid garter snakes is well established (Rossman et al. 1996). In my study, I held all snakes in similar outdoor enclosures for most of gestation and moved them to indoor holding tanks when they were expected to give birth. The environmental conditions that the snakes experienced in the outdoor enclosures were probably very similar to those they would have experienced in nature. Indoors, the temperatures of the holding rooms were allowed to fluctuate with environmental conditions but were maintained above 20°C, and most of the snakes were provided with a heat source (heating

Table 1-4. Effects of time in captivity, date of birth and year of study on the presence and number of stillborn young in garter and ribbon snake litters. Presence of stillborn young was analysed in a multiple logistic regression against the independent variables. Number of stillborn young (in those litters containing stillborn young) was analysed in a multiple linear regression against the independent variables.

Species	Variable	Presence of stillborn young				Number of stillborn young			
		χ^2	p	R ²	n	F	df	p	R ²
Garter Snakes	Overall analysis	21.33	0.0003	0.22	70	2.70	3,31	0.06	0.21
	Time in captivity	0.63	0.43			3.60		0.07	
	Date of birth	10.07	0.002			6.32		0.02	
	Year of study	6.34	0.04			5.74		0.02	
Ribbon Snakes	Overall analysis	12.03	0.02	0.21	42	1.06	4,13	0.41	0.25
	Time in captivity	1.37	0.24			0.43		0.52	
	Date of birth	5.37	0.02			2.37		0.15	
	Year of study	5.30	0.07			2.08		0.16	

rock or external incandescent light bulb) for thermoregulation. However, in 1998, some of the snakes did not have access to a heat source in their holding tank, and two different types of heat sources were provided in 1999. When I compared 1999 females with different heat sources, I found that those females with constant access to heating rocks in late gestation did not have a lower chance of giving birth to stillborn young than those who were provided with intermittent radiation from light bulbs (GS: Fisher Exact Test, $p=0.17$, $n=18,16$; RS: Fisher Exact Test, $p=1.00$, $n=19,7$). Furthermore, the number of stillborn young in those litters containing stillborns did not differ between the two captive conditions (GS: Mann-Whitney test, $S=39.5$, $p=0.23$, $n=6,10$; RS: Mann-Whitney test, $S=5.5$, $p=0.23$, $n=7,2$). The incidence of stillbirths seemed to decrease from 1998 to 1999, when all females had access to external heat sources during late gestation, but the number of litters containing stillborn young was still high (16 of 34 garter snake litters and 9 of 26 ribbon snake litters). Perhaps, even though the heat sources were present, some females did not use them for thermoregulation given the artificial nature of their captive conditions. If conditions in captivity were poorer than natural conditions, I would have also expected to see that females did not feed well and, therefore, did not grow during my study. However, most females fed quite well while in captivity (personal observation). Furthermore, the SVL of the captive snakes did increase significantly from the time of capture until the time of parturition (when they were released) in both species (GS: paired t-test, $t=6.41$, $p<0.0001$, $n=68$; RS: paired t-test, $t=4.48$, $p=0.0001$, $n=42$), indicating that the snakes received enough resources to produce a litter and to invest in their own growth.

Because stillborn young in northern water snakes were predominantly male (Weatherhead et al. 1998), I determined whether male or female young were more likely to be stillborn in my study species. In garter snakes, 130 of 441 female neonates (29.5%) and 122 of 445 male neonates (27.4%) were stillborn. In ribbon snakes, 31 of 196 female neonates (15.8%) and 25 of 186 male neonates (13.4%) were stillborn. Therefore, there was no difference in the probability that male and female offspring were stillborn in garter or ribbon snakes (Fisher Exact Test, $p=0.50$, $p=0.56$, respectively). Next, I determined whether the offspring in litters with stillborn young differed in size from those in litters without stillborn young (Table 1-5). The median SVL of offspring in litters containing stillborn young did not differ from that in litters without stillborn young in garter or ribbon snakes (GS: ANOVA, $F_{1,65}=0.07$, $p=0.79$, $r^2=0.001$, $n=32, 35$; RS: ANOVA, $F_{1,39}=0.09$, $p=0.76$, $r^2=0.002$, $n=17, 24$). Median neonate mass was significantly lower in litters with stillborn young in garter snakes (ANOVA, $F_{1,68}=11.30$, $p=0.001$, $r^2=0.14$, $n=35, 35$), but not in ribbon snakes (ANOVA, $F_{1,40}=0.61$, $p=0.44$, $r^2=0.01$, $n=18, 24$). However, stillborn neonates weighed significantly less than live young in both garter and ribbon snakes overall (Mann-Whitney test, $p<0.0001$, $p=0.006$, respectively).

In summary, I have found that the presence of stillborn young did not depend on maternal size, litter size or time in captivity, although an increase in date of parturition did correspond to an increase in the probability of stillbirths. I also showed that male and female embryos were equally susceptible to mortality during development. Lastly, stillborn young weighed less than live young, but only garter snake litters with stillborn young differed from litters without stillborn young in median neonate mass. Because a female's

Table 1-5. Comparison of median neonate size for litters with and without stillborn young in garter and ribbon snakes. Values represent means of median measurements for all neonates in the litter, and sample sizes are indicated in parentheses. Values were compared by ANOVA, and p-values are presented.

	Garter snake litters			Ribbon snake litters		
	with stillborn	without stillborn	p-value	with stillborn	without stillborn	p-value
SVL (cm)	14.54 (32)	14.59 (35)	0.79	15.00 (17)	14.93 (24)	0.76
mass (g)	1.56 (35)	1.81 (35)	0.001	1.39 (18)	1.45 (24)	0.44

investment of resources into stillborn young is wasted, the presence of stillborn young in her litter represents a considerable cost and could not result from an adaptive strategy. For this reason, I have concluded that the high occurrence of stillborn young seems to be an artifact of my study and I have conducted the subsequent analyses twice: once with all litters, and once without those litters that contained stillborn young. The results of the latter analyses are presented only when they differ qualitatively from the former. Overall, the sex ratios of litters with stillborn young did not differ significantly from those without stillborn young in both species (GS: t-test, $t=0.234$, $p=0.80$, $n=70$; RS: t-test, $t=1.040$, $p=0.30$, $n=42$).

Adult sexual size dimorphism

My prediction that litters born to large females and litters containing large offspring would be female-biased was based on the assumption that females should benefit more from large size at birth because size dimorphism is female-biased in both species. To confirm that females are larger than males in my study population, I compared measurements from all adult male and female snakes brought into captivity (Table 1-6). As adults, females are both longer (Mann-Whitney test, $S=33339$, $p<0.0001$, $n=145$, 208) and heavier (Mann-Whitney test, $S=36474.5$, $p<0.0001$, $n=147$, 207) than males in this garter snake population (Figure 1-1). Because gravid females necessarily weigh more than other females, I compared the masses of males and females after excluding all females captured later than May, when females are thought to ovulate and begin gestation. Still, adult females weighed more than adult males (Mann-Whitney test, $S=23545$, $p<0.0001$, $n=105$,

Table 1-6. Comparison of size measurements of male and female adult garter and ribbon snakes at time of capture. Values are reported as means, and sample sizes are presented in parentheses. P-values from Mann-Whitney tests comparing males and females are also reported.

	Garter Snakes		Ribbon Snakes	
	Male	Female	p-value	p-value
SVL (cm)	41.9 (208)	47.6 (145)	<0.0001	<0.0001
Mass (g)	35.0 (207)	69.3 (147)	<0.0001	<0.0001
Mass (g) - non gravid females ¹	35.0 (207)	65.0 (105)	<0.0001	<0.0001
Tail (proportion of SVL)	0.30 (208)	0.26 (145)	<0.0001	<0.0001
			Male	Female
			47.4 (53)	39.8 (62)
			25.0 (53)	50.4 (62)
			25.0 (53)	48.8 (32)
			0.46 (53)	0.39 (62)

¹ measurements of mass for females captured later than 31 May were excluded from analysis

Figure 1-1. Relationship between SVL and mass of female and male adult garter snakes (n=145, 207). Females were both longer and heavier than males (Mann-Whitney test, $p < 0.0001$).

207). In ribbon snakes, adult females are also longer (Mann-Whitney test, $S=1904.5$, $p<0.0001$, $n=62, 53$) and heavier (Mann-Whitney test, $S=1682$, $p<0.0001$, $n=62, 53$) than adult males (Figure 1-2). After removing females captured after 31 May, the difference in mass was still highly significant (Mann-Whitney test, $S=2116$, $p<0.0001$, $n=32, 53$).

Therefore, sexual size dimorphism is female-biased in both garter and ribbon snakes.

Sexual dimorphism at birth

With evidence of sexual size dimorphism in adults of both species, I determined whether dimorphism existed at birth (Table 1-7). When all garter snake offspring were considered together, male and female young did not differ significantly in SVL (Mann-Whitney test, $S=139236.5$, $p=0.72$, $n=368, 382$) or in mass (Mann-Whitney test, $S=195753.5$, $p=0.92$, $n=444, 441$). Similarly, within a litter, there were no differences between male and female young in SVL (paired t-test, $t=0.17$, $p=0.86$, $n=61$) or in mass (paired t-test, $t=0.41$, $p=0.68$, $n=69$).

Among all ribbon snake neonates, males were significantly longer (Mann-Whitney test, $S=35069$, $p=0.02$, $n=191, 177$) and heavier than females (Mann-Whitney test, $S=38240$, $p=0.02$, $n=196, 186$). For only live young, males and females did not differ in SVL (Mann-Whitney test, $S=27532.5$, $p=0.16$, $n=161, 165$), but males were significantly heavier than females (Mann-Whitney test, $S=28029.5$, $p=0.04$, $n=161, 165$). Within litters, neither SVL (paired t-test, $t=1.46$, $p=0.15$, $n=41$) nor mass (paired t-test, $t=0.68$, $p=0.50$, $n=42$) differed between male and female young. Because male ribbon snake neonates were larger than females overall, but size did not differ between sexes within litters, it suggests that neonates in male-biased litters were larger (see below).

Figure 1-2. Relationship between SVL and mass of female and male adult ribbon snakes (n=62, 53). Females were both longer and heavier than males (Mann-Whitney test, $p < 0.0001$).

Sex ratio and neonate characteristics

Because adult females are larger than adult males in both garter and ribbon snakes, females should benefit more than males from large size at birth. However, female and male young did not differ in size in garter snakes. Therefore, garter snakes do not seem to bias the sex ratio of their litters with neonate size. Thus, I have investigated the relationship between litter sex ratio and neonate size only in ribbon snakes. I found that sex ratio was not significantly related to median neonate SVL (ANCOVA, $F_{3,37}=2.46$, $p=0.08$, $R^2=0.17$). However, litter sex ratio did increase as median neonate mass increased (ANCOVA, $F_{3,38}=2.83$, $p=0.05$, $R^2=0.18$; year: $F=0.81$, $p=0.45$; mass: $F=4.85$, $p=0.03$). After litters with stillborn young were removed, the relationship between median neonate mass and sex ratio in all years of study was no longer significant (ANCOVA, $F_{3,20}=0.74$, $p=0.54$, $R^2=0.10$). In the latter analysis, which was restricted to litters with only live young, the direction of the relationship between sex ratio and neonate size was the same, and the lack of significance might have reflected a lack of power because of the smaller sample size rather than indicating no relationship between the variables. In univariate analyses of ribbon snake litters, sex ratio increased with both median neonate SVL (Figure 1-3; linear regression, $F_{1,39}=4.36$, $p=0.04$, $r^2=0.10$) and median neonate mass (Figure 1-4; linear regression, $F_{1,40}=6.95$, $p=0.01$, $r^2=0.15$). Elsewhere, I have shown that neonate SVL and mass were highly correlated in ribbon snakes (Chapter 2). Because of this correlation, a similar relationship between sex ratio and neonate SVL or neonate mass is not surprising.

Figure 1-3. Relationship between litter sex ratio (SR) and median neonate SVL (NS) in ribbon snakes. The proportion of males in a litter increased as neonate SVL increased ($r^2=0.10$, $p=0.04$, $n=41$).

Figure 1-4. Relationship between litter sex ratio (SR) and median neonate mass (NM) in ribbon snakes. The proportion of males in a litter increased as neonate mass increased ($r^2=0.15$, $p=0.01$, $n=42$).

Sex ratio and maternal characteristics

Although I did not conduct any analyses of sex ratio with neonate size in garter snakes, I have included both species in my analyses of sex ratio with maternal size, because maternal and neonate size can influence sex ratio independently (Weatherhead et al. 1998). However, when controlling for year, the relationship between sex ratio and maternal SVL was not significant in garter or ribbon snakes (GS: ANCOVA, $F_{3,66}=1.72$, $p=0.17$, $R^2=0.07$; RS: ANCOVA, $F_{3,38}=1.18$, $p=0.33$, $R^2=0.09$). When considered together, neither maternal SVL nor maternal mass explained a significant amount of variation in sex ratio in garter or ribbon snakes (GS: ANCOVA, $F_{4,65}=1.28$, $p=0.29$, $R^2=0.07$; RS: ANCOVA, $F_{4,37}=0.97$, $p=0.43$, $R^2=0.10$).

I have shown elsewhere that neonate SVL and mass increased significantly with maternal SVL in both garter and ribbon snakes (Chapter 2). To determine the combined effects of maternal and neonate size on sex ratio, I considered both size measurements simultaneously. In garter snakes, maternal SVL and neonate SVL did not explain a significant amount of variation in sex ratio (ANCOVA, $F_{4,62}=1.91$, $p=0.12$, $R^2=0.11$). In ribbon snakes, sex ratio increased with neonate SVL, but was not related to maternal SVL (ANCOVA, $F_{4,36}=2.57$, $p=0.05$, $R^2=0.22$; neonate SVL: $F=6.08$, $p=0.02$; maternal SVL: $F=2.58$, $p=0.12$; year: $F=1.43$, $p=0.25$). In both species, the relationship of sex ratio with maternal SVL tended to be negative, while the relationship with neonate SVL was positive.

Sex ratio and date of birth

In garter and ribbon snakes, sex ratio did not vary significantly with birth date when controlling for year (GS: ANCOVA, $F_{3,66}=2.06$, $p=0.11$, $R^2=0.09$; RS: ANCOVA, $F_{3,38}=1.27$, $p=0.30$, $R^2=0.09$). The results of these analyses did not differ qualitatively when date of birth of a litter was considered relative to other litters' dates of birth in that year.

Discussion

As was predicted from sex ratio theory, the overall natal sex ratio and the mean litter sex ratio of garter and ribbon snakes did not differ significantly from equality. I also tested the prediction that females adaptively adjusted the sex ratio of their litters in response to their own size and to the size of their young. I found no evidence for this pattern of adaptive sex allocation in my study species. In fact, in ribbon snakes, there was some evidence of an increase in sex ratio as neonate size increased, instead of the expected decrease. Furthermore, the negative relationship between sex ratio and maternal size was not significant in both species. Lastly, variation in date of parturition did not contribute significantly to variation in sex ratio in either study species.

My failure to find the expected pattern of sex ratio variation in both of my study species may be due in part to the patterns of sexual size dimorphism at birth. I did verify that adult garter and ribbon snakes were sexually dimorphic in body size. In both species, females grew significantly longer and heavier than males. Although females should benefit more than males from any factor (like large size at birth, or earlier birth date) that allows

them to reach adult size sooner, female neonates were not larger than males in garter snakes. Because they are the same size at birth, male and female offspring represent equal costs to their mother. Thus, an adult garter snake would not be expected to vary the sex ratio of her litter with neonate size. In ribbon snakes, the size of male neonates was greater than that of female neonates, but there was no significant difference in size between the sexes within litters. The presence of an overall difference in size between male and female neonates accompanied by the lack of such a difference within litters in ribbon snakes suggests that all offspring within male-biased litters might be larger. Indeed, I found that ribbon snake litters with heavier offspring were more male-biased, and litters with lighter offspring were more female-biased. In this species, the increase in sex ratio with offspring size was opposite to the relationship expected based on the pattern of sexual size dimorphism in adults.

The second prediction that I tested was that sex ratio would become more female-biased as maternal size increased, because larger females could invest more resources into their presumably more costly female offspring. However, the relationship between maternal size and sex ratio was not significant in garter or ribbon snakes. More importantly, I have shown that, although maternal size and neonate size are positively correlated, the relationship between maternal size and sex ratio is not necessarily present when the relationship between neonate size and sex ratio exists. However, in two studies of litter sex ratio variation, sex ratio was assumed to vary with neonate size because it varied with maternal size (Lemen and Voris 1981, Dunlap and Lang 1990). My data suggest that the assumption of an effect of neonate size on sex ratio without its direct

measurement may have led to erroneous conclusions in those studies. A more recent study compared sex ratio and neonate size directly and showed that litters became more female-biased as neonate size increased, although maternal and neonate size were found to influence sex ratio independently of each other (Weatherhead et al. 1998). In my study of ribbon snakes, there was no relationship between sex ratio and maternal size, although sex ratio became more male-biased as neonate size increased. This relationship does conform to the expectation that litters with larger young will be biased towards the sex that is born larger. However, the advantage to males being born larger in a species where sexual size dimorphism is female-biased is questionable. Obviously, additional investigation of the relationship between sex ratio and neonate size in snakes is necessary to demonstrate and understand the existence of adaptive sex ratio variation.

Lastly, I attempted to determine whether variation in date of birth accounted for variation in sex ratio. Only one study has shown that sex ratio varied predictably with date of birth (Osgood 1978). In that study, female water snakes, *N. fasciata*, maintained at higher temperatures gave birth to more female-biased litters than females maintained at lower temperatures (Dunlap and Lang 1990). Weatherhead et al. (1998) reasoned that those females held at higher temperatures would have given birth to their female-biased litters earlier than the low-temperature females gave birth to their male-biased litters. However, they recognised that these skewed sex ratios could be explained by the non-adaptive, differential mortality of males and females during embryonic development as a function of incubation temperatures, a phenomenon that has been shown in pine snakes, *Pituophis melanoleucus* (Burger and Zappalorti 1988). Thus, the sex ratio variation

observed in *N. fasciata* can not be used as evidence of adaptive sex allocation.

Additionally, in *N. sipedon*, litter sex ratios did not vary with date of birth because females could not predict the thermal conditions in which they would be held during gestation at the time of ovulation (Weatherhead et al. 1998). In my study, I predicted that females would adjust the sex ratio of their litter in response to the timing of ovulation. Although variation in date of parturition was more likely the result of variation in date of ovulation than variation in conditions during gestation, I did not find a significant relationship between sex ratio and date of birth in either garter or ribbon snakes.

As pointed out previously, many young were stillborn in my study, but their occurrence did not seem to influence the conclusions drawn in my study. First, there was no difference in the probability that male or female offspring would be stillborn. Previously, Weatherhead et al. (1998) found that the stillborn young in their study of water snakes were predominantly male. They suggested that this phenomenon might indicate that male embryos were more sensitive than female embryos to the high temperatures obtained by gravid females while they were thermoregulating. I found no evidence of such a difference in susceptibility in my study species. More importantly, an exclusion of the litters with stillborn young did not change the results of any of my analyses qualitatively, indicating that litters with stillborn young did not differ significantly from those litters with only live young. For these reasons, it seems reasonable to believe that similar patterns of sex ratio variation would exist in the absence of stillborn offspring.

Although the occurrence of such a high number of stillborn young in my study did not have an overall effect on my results, it was clearly non-adaptive and merits further

investigation. I have suggested that the presence of stillborn young in a litter might depend on several factors, including maternal size, litter size, time in captivity, and date of birth. However, the probability that a litter contained stillborn young was not related to maternal size or the number of offspring in the litter. In both garter and ribbon snakes, the probability of a litter containing stillborn young increased with date of birth. Furthermore, in garter snakes, females who gave birth later in the season seemed to give birth to more stillborn young. In previous studies, the length of time that a female was held in captivity had a negative effect on her litter characteristics by increasing the chance and/or number of stillborn young in the litter (Farr and Gregory 1991). An increase in stillborn young with increasing date of birth could have indicated a negative effect of captivity in my study. However, when time in captivity was included in the analyses, it did not contribute to the probability or the number of stillborn young in a litter in either garter or ribbon snakes. I recognise that the high number of stillbirths during my study is unusual and represents an enormous cost to snakes. Although I did not determine what factors contributed to the high rate of stillbirths during my study, it is likely an artifact of my study. It seems that a study needs to be undertaken where variables during gestation, like opportunity for thermoregulation and amount of food available, are manipulated for all gravid females. The comparison of the occurrence of stillbirths among females in each treatment might reveal some causative factors of stillbirths in these populations of garter and ribbon snakes.

Overall, my results revealed two different patterns of sex ratio variation in two closely-related and coexisting species of snakes, which are added to the existing body of

inconsistent evidence from four previous studies. Initially, I recognised that there were some inadequacies in the studies performed to date. Most importantly, two of the studies had not measured offspring size directly, but assumed that a relationship between offspring size and sex ratio existed when sex ratio was shown to depend on maternal size (Lemen and Voris 1981, Dunlap and Lang 1990). As outlined previously, I have shown that such an assumption may have been invalid. In both garter and ribbon snakes, offspring size was found to be correlated to maternal size. However, in ribbon snakes, sex ratio increased as neonate size increased and did not vary predictably with maternal size. Another weakness of those two studies may have been that they relied on data from embryos of sacrificed snakes. Because of their experimental procedure, many measurements (such as offspring size, date of birth and number of stillborn young) could not be estimated. Furthermore, it seems reasonable to question how accurately they could determine the sex of a female's offspring, given that they were removed during embryological development. Only one of the previous studies has documented the sex ratios of litters after parturition and has compared them to neonate size directly (Weatherhead et al. 1998).

Although one of the aims of my study was to elucidate the patterns of sex ratio variation in snakes by examining the characteristics of garter and ribbon snake litters, I found insufficient evidence to suggest that all snakes adjust the sex of their offspring adaptively. In fact, I found that sex ratio varied non-randomly only in ribbon snakes, and that this variation occurred opposite to the expected direction, given that sexual size dimorphism was female-biased in this species. Added to previous studies' findings of random sex ratio variation in one species (*N. rhombifer*), and non-random sex ratio

variation consistent with adaptive sex allocation theory in two species (*E. schistosa* and *N. sipedon*) and opposite to Trivers-Willard (1973) theory in another species (*T. sirtalis*), my results suggest that the evidence for adaptive sex ratio variation in snakes remains ambiguous. There is still a need for additional studies of litters from numerous snake species before conclusions can be drawn as to whether sex ratios vary in accordance with Trivers and Willard's (1973) adaptive sex allocation theory.

CHAPTER 2:

Patterns of offspring size variation in eastern garter snakes, *Thamnophis sirtalis sirtalis*, and northern ribbon snakes, *Thamnophis sauritus septentrionalis*

Introduction

Many life history studies address the issue of how a female should allocate resources between her current litter and her own growth, survival and future reproduction (Stearns 1992). Of equal interest is the problem of how females should manage the trade-off between offspring size, quality and number (Roff 1992). Smith and Fretwell (1974) proposed that for a given population there is an optimal size for offspring and that females maximise their fitness by investing resources into offspring of that size. Thus, a female with more energy to invest should produce more offspring of the "optimal" size rather than increasing the size of some or all of her offspring. Although the concept of an optimal offspring size within populations is well-established in the life history literature, there is a great deal of evidence that offspring size varies substantially within populations in many taxa (Roff 1992, Stearns 1992, Bernardo 1996a). Such variation is well documented in snakes, both within and among litters (Ford and Killebrew 1983, Brodie and Ducey 1989, Seigel and Ford 1992, King 1993, Larsen et al. 1993, Madsen and Shine 1996, Weatherhead et al. 1999). Despite numerous attempts to investigate the relationships between offspring size and various reproductive characteristics within species, no overall explanation of offspring size variation has been proposed. However, it has been suggested that the application of the optimal offspring size theory is inappropriate in snakes and should be replaced by an examination of the factors

influencing offspring size variation within populations (Weatherhead et al. 1999). In the present study, I have gathered data on two snake species, *Thamnophis sirtalis sirtalis* and *T. sauritus septentrionalis*, to quantify the variation in offspring size and to determine the factors that contribute to that variation. I also have attempted to explain why offspring size is variable in snakes, contrary to predictions from the optimal offspring size theory.

In many snake species, investment by a female in her litter ends at parturition (Clutton-Brock 1991). Thus, there are limited ways in which a female can influence the fitness of her young. In Chapter 1, I discussed the influence that a female has over the sex ratio of her litter. Here, I investigate how a female allocates energy into offspring. Previous studies have shown that offspring size varies with maternal size, litter size, and date of birth. Thus, much of the variation in offspring size can be attributed to maternal effects, which encompass any effect of a female's phenotype on the fitness of her young above and beyond her contribution to their genotypes (Bernardo 1996a, 1996b). For instance, in many taxa, offspring size increases with maternal size (Roff 1992). Sometimes, this variation results from physical limitations that prevent small females from producing offspring as large as the optimal offspring size. For example, in two species of turtles, egg width was limited to a less than optimal size because of the size of a female's pelvic aperture, which was restricted by other selection pressures (Congdon and Gibbons 1987). Consequently, offspring size is expected to increase with maternal size, until females are large enough to produce young of the optimal size. In numerous snake species, offspring size increased with maternal size (Ford and Killebrew 1983, Ford and Karges 1987, King 1993). However, because these relationships can not be explained by the physical limitations of the mother, they most likely

represent allocation decisions by the mother, where a larger female can afford to invest more energy in each of her offspring. Thus, I have investigated the direct relationship between maternal and offspring size in garter and ribbon snakes.

The effects of maternal size on neonate size might not be limited to direct effects, and might also occur through a female's control of litter size. The number of offspring in a litter increases with maternal size in many snake species (Seigel and Ford 1987). As stated previously, optimal offspring size theory predicts that females should produce different-sized litters of equally-sized offspring (Smith and Fretwell 1974). However, in some snake populations, females produced litters along a continuum from small litters of large neonates to larger litters of small neonates (King 1993, Madsen and Shine 1996, Weatherhead et al. 1999). In contrast, there was no relationship between offspring size and number in other snakes (Brodie and Ducey 1986, Ford and Seigel 1989, Seigel and Ford 1991, Gregory and Larsen 1993, Greene et al. 1999). Because the current evidence is inconclusive, I examined the relationship between litter size and offspring size in my study species.

Some variation in offspring size can also be explained by the "fractional offspring" hypothesis, which predicts that a female with additional resources should produce more offspring of a less than optimal size or fewer offspring of a greater than optimal size (Ricklefs 1968). There is evidence from two species of reptiles, that when a female's resources are insufficient to increase litter size, they are divided among existing offspring, resulting in variability in offspring size (Nussbaum 1981, Schwarzkopf 1992). Of course, the effect of a "fractional offspring" on offspring size variation should be greatest in organisms

that produce small litters (Ebert 1994), so I would expect that the among-litter variance of offspring size should decrease as litter size increased.

In a study of *Thamnophis marcianus*, litter size differed significantly between females in a high-energy treatment and those in a low-energy treatment, although among-female variance in offspring size did not differ between treatments (Seigel and Ford 1992). This suggests that larger litters (i.e. high energy) were not significantly less variable than smaller litters (i.e. low energy). Thus, the fractional-offspring hypothesis did not account for the observed variation in offspring size. In the same study, within-female variance in offspring size did differ between treatments, but variance in offspring size was not significantly related to female SVL or litter size. However, there is some evidence that maternal effects can influence the variability of offspring size (Bernardo 1996a). Thus, I have also investigated the variation in neonate size with respect to maternal size and litter size.

Females could also benefit from adjusting the size of their offspring in relation to the timing of their birth. Nussbaum (1981) showed that offspring size in the side-blotched lizard, *Uta stansburiana*, increased as date of parturition increased. He suggested that this trend resulted from females compensating for late birth date by investing more energy per offspring. Because parturition occurs late in the season in my study species, those young born later have less time to forage before entering hibernation and should benefit more from large size at birth than young born earlier. In snakes, females have the potential to control timing of parturition by varying the timing of ovulation and by using behavioural thermoregulation to effect the duration of gestation. Females who devote more time to

thermoregulatory activities can accelerate the development of their young and give birth earlier. Thus, females should adjust their investment into offspring size in relation to their expected timing of parturition. Although this pattern was not observed in northern water snakes, *Nerodia sipedon* (Weatherhead et al. 1999), offspring born later were larger in the brown snake, *Storeria dekayi* (King 1993). Thus, I examined whether offspring size increased with date of birth in garter and ribbon snakes.

In two previous studies of snakes, larger litters were born earlier (King 1993, Weatherhead et al. 1999). In both studies, larger litters were also produced by larger females. Thus, Weatherhead et al. (1999) suggested that the relationship between litter size and date of parturition could be caused by differences in either thermal inertia or ovulation dates. The first explanation suggests that, because of their larger size, larger females are better able to maintain their body temperature at an optimal level for embryonic development, thus giving birth to their larger litters earlier. Alternatively, larger females might acquire sufficient energy for ovulation sooner. Given similar environmental conditions for all females, this would result in larger litters being born earlier. Because both explanations could apply to my study species, I have investigated the relationship between parturition date and litter size.

If offspring have some control over the allocation of energy that they receive from their mother, they could invest more energy into growth at the expense of keeping energy in reserve. In scincid lizards, *Eulamprus heatwolei*, neonates that developed more quickly and were born earlier (due to higher temperatures during gestations) were longer-bodied at birth (Shine and Harlow 1993). This was likely a result of those neonates putting more energy

into growth and keeping less in reserve. Conversely, neonates that developed more slowly and were born later were shorter in SVL and kept more energy in reserve. Because garter and ribbon snake neonates born later in the season have less time to forage before entering hibernation, they should benefit more than neonates born earlier from keeping energy in reserve instead of investing it into length. Although the expected pattern was not seen in water snakes (Weatherhead et al. 1999), I did investigate the effect of date of parturition on offspring SVL.

The importance of SVL at birth was shown in Fitch's garter snake, *Thamnophis sirtalis fitchi*, where longer neonates were found to survive better (Jayne and Bennett 1990). However, offspring quality is often traded off against offspring number and size (Roff 1992). Thus, studies of offspring quality are not limited to measurements of offspring size. For example, number of vertebrae displayed a moderate to high heritability within populations (Arnold 1988), and an increase in the number of vertebrae corresponded to an increase in locomotory ability in garter snakes (Dohm and Garland 1993). An increase in locomotory performance often results in successful evasion of a predator and, thus, an increase in survival (Jayne and Bennett 1990). Because larger females should be able to invest more energy in the quality of their young, I expect that their offspring will have more vertebrae and greater locomotory ability than neonates born to smaller females.

Materials and Methods

I conducted this study from April to September of 1997 to 1999 at the Queen's University Biological Station (QUBS) in eastern Ontario, Canada (45° 37' N, 76° 13' W). As

outlined in Chapter 1, I relied on opportunistic sightings of garter and ribbon snakes in forests, marshes, fields and roadways on QUBS property, and attempted to hand-capture every snake that was seen. Other researchers at QUBS also caught snakes they saw, and brought them into captivity. In the lab, I recorded total length, tail length, snout-to-vent length (SVL), and mass of each snake within 12 hours of capture as described in Chapter 1. I implanted a transponder under the skin of adult female snakes to ensure accurate identification over the course of the study.

I described the housing conditions of snakes from the time of capture until parturition in Chapter 1. At parturition, I collected all neonates and follicles for measurement and recorded date of parturition as Julian date. I measured the SVL and mass of each adult female within 24 hours of parturition and recorded the number of neonates (live and stillborn) in her litter. I measured SVL, tail length, mass and sex for each neonate (live and stillborn) within 24 hours of parturition. I determined neonate SVL and tail length (to the nearest 0.1 cm) by placing the neonate directly on a ruler, and measured mass to the nearest 0.1 g. I determined the sex of each neonate by probing the cloaca for the presence of hemipenes. I recorded the litter mass as the total mass of all neonates (live and stillborn) as well as follicles. Relative clutch mass (RCM) was calculated by dividing litter mass by a female's mass after parturition. As a measure of the number of vertebrae of each neonate, I recorded the total number of ventral scales. To represent locomotory performance, I measured swimming performance of neonates in a 2 m trough within 48 hours of birth. Speed was measured as the time it took an individual to swim the length of the track. Two

trials were performed with each neonate, and the quicker of the two trials was taken to represent its burst speed.

Statistical analyses

When my data did not meet the assumptions of parametric tests, I transformed measurements of maternal size and time in captivity with log transformations, and litter size with square-root transformations. I used non-parametric tests of the untransformed data when the assumptions were not met. Because the size measurements of neonates within a litter were not normally distributed, I used median values of SVL and mass to represent a litter's neonate measurements. As described previously, I used SVL of a female after parturition to represent maternal SVL to reduce variation in measurements due to timing of capture. I did verify that, although SVL increased from time of capture to parturition, both measures of SVL were highly correlated in garter and ribbon snakes (Chapter 1). Similarly, I used a female's post-partum mass, instead of mass at capture, to represent maternal mass, because some females were captured before gestation began while others were gravid at the time of capture.

To compare within-litter variance in offspring size to maternal size and litter size, I used the statistical method outlined by Sokal and Braumann (1980) for comparing coefficients of variation (CV) among samples. This involved calculating the deviations of each measurement from the mean of the variable as $\left| \ln Y_{ij} - \overline{\ln Y_i} \right|$, where $\ln Y_{ij}$ is the logarithm of offspring size of the j^{th} individual in the i^{th} litter and $\overline{\ln Y_i}$ is the mean of the logarithms for that litter. For each litter, I determined the median of these deviations

(because they were not normally distributed within litters) and compared them (with Pearson product-moment correlations) to maternal size and litter size.

I used both univariate and multivariate analyses to examine the relationships in my study. I used univariate analyses to identify and quantify the direct effects of the independent variables on variation in offspring SVL and offspring mass, as well as the relationship between maternal size and other indices of offspring quality. For multivariate analysis, I used path analysis, which allowed me to assess the relative importance of multiple independent variables on multiple dependent variables (Kingsolver and Schemske 1991). Because path analysis considers both direct and indirect effects of independent variables on each dependent variable, it is favoured over multiple regressions or partial correlations. For my path analysis, I generated a diagram based on the hypothesised relationships between variables. The strength of the non-causal correlation between two variables (eg. maternal SVL and maternal mass) was indicated by the Pearson product-moment correlation coefficient. A direct, causal effect of an independent variable on a dependent variable was represented by a one-way arrow between two variables. The strength of each effect was determined by the magnitude of the standardised partial-regression coefficient from a multiple regression of the dependent variable on its set of independent variables. For example, the path coefficient between maternal SVL and parturition date represents the direct effect of maternal SVL on parturition date with all other independent variables (maternal mass, litter size and capture date) held constant. The strength of indirect effects was calculated as the product of the direct effects between the variables of interest. For example, the indirect effect of capture date on time in captivity is the product of the direct

effect of capture date on parturition date and the direct effect of parturition date on time in captivity. The overall effect of one variable on another was the sum of its direct and indirect effects. I have indicated the statistical significance of each path coefficient to show the relative importance of the direct effects in the path diagram. However, the lack of statistical significance of path coefficients was not used to assess the accuracy of the path diagram, because the importance of indirect effects between variables would have been ignored (Kingsolver and Schemske 1991). Lastly, residual variables, indicated by U, represented the unexplained variance in the multiple regression for each dependent variable and were calculated as $(1-R^2)^{0.5}$

I designed my path analysis to assess direct effects of maternal SVL, maternal mass (where differences in mass for a given SVL represent variation in maternal condition), litter size, date of parturition and time in captivity on neonate mass. Neonate SVL should be directly affected by neonate mass, with a potential effect of parturition date (if offspring can control energy allocation during development) and time in captivity. I included time in captivity in my analyses because females were held in captivity for different amounts of time before parturition, and captivity is known to influence the characteristics of neonates in snakes (Farr and Gregory 1991). By definition, time in captivity is determined by capture date and parturition date. If the effects of captivity on a female's litter are negative, offspring mass and offspring SVL should decrease as time in captivity increases. Based on the hypothesised relationships between variables in my study, I conducted the following multiple regression analyses: (1) litter size regressed on maternal SVL and maternal mass; (2) parturition date regressed on maternal SVL, maternal mass and capture date; (3) time in

captivity regressed on parturition date and capture date; (4) neonate mass regressed on maternal SVL, maternal mass, litter size, parturition date and time in captivity; and (5) neonate SVL regressed on neonate mass, parturition date and time in captivity.

Because date of birth varied significantly among years (Chapter 1), I conducted all of my analyses with relative date of birth to correct for variation among years, but only reported these results when they differed qualitatively from the analyses with absolute date of birth. I conducted all analyses separately for garter and ribbon snakes using JMP (version 3.1) statistical software (SAS Institute Inc. 1994), and accepted significance at 0.05 level.

Results

As demonstrated elsewhere, there was a great deal of variation in maternal, litter and neonate characteristics during my study (Chapter 1). Most importantly, neonate size was highly variable. Overall, neonate SVL ranged from 7.6 to 16.8 cm in garter snakes and from 9.0 to 17.2 cm in ribbon snakes. Neonate mass ranged from 0.1 to 2.8 g in garter snakes and from 0.8 to 2.2 g in ribbon snakes. The smallest garter snake neonate to survive at least until it was released weighed 0.7 g, and the smallest ribbon snake was 0.8 g. Among litters, median neonate SVL ranged from 12.7 to 16.3 cm in garter snakes and from 13.6 to 17.0 cm in ribbon snakes. Median values of neonate mass ranged from 1.0 to 2.4 g in garter snakes and from 1.0 to 2.0 g in ribbon snakes. Even within litters, there was some variation in neonate size. Considering only neonates that were born alive and survived at least until they were released, the largest range of SVL measurements within a litter of garter snakes was from 11.4 to 16.4 cm, and the largest range of neonate masses was 0.7 to 2.1 g. In

ribbon snake litters, the largest range of neonate SVL was 9.0 to 16.0 cm, and of neonate mass was 0.8 to 1.8 g.

Offspring size

In garter snakes (GS) and ribbon snakes (RS), median neonate SVL increased with median neonate mass (Figure 2-1; GS: linear regression, $F_{1,65}=84.63$, $p<0.0001$, $r^2=0.57$; RS: linear regression, $F_{1,39}=82.36$, $p<0.0001$, $r^2=0.68$). Because neonate SVL and mass are highly correlated in both study species, and because offspring mass is more directly influenced by maternal size, I have used offspring mass in all subsequent univariate analyses of offspring size.

Effects of maternal size and litter size on offspring size

In garter and ribbon snakes, longer females gave birth to larger offspring (Figure 2-2; GS: linear regression, $F_{1,68}=16.05$, $p=0.0002$, $r^2=0.19$; RS: linear regression, $F_{1,40}=4.15$, $p=0.05$, $r^2=0.09$) and to larger litters (Figure 2-3; GS: linear regression, $F_{1,68}=14.12$, $p=0.0004$, $r^2=0.17$; RS: linear regression, $F_{1,40}=6.64$, $p=0.01$, $r^2=0.14$). Consequently, the trade-off between offspring size and number was not significant in garter or ribbon snakes (GS: linear regression, $F_{1,68}=0.12$, $p=0.73$, $r^2=0.002$; RS: linear regression, $F_{1,40}=0.33$, $p=0.57$, $r^2=0.008$). When I controlled for differences in maternal SVL, larger litters were composed of smaller neonates in garter snakes (multiple linear regression, $F_{2,67}=11.26$, $p=0.0001$, $R^2=0.25$; maternal SVL: $F=22.36$, $p<0.0001$; litter size: $F=5.42$, $p=0.02$). However, offspring mass and number were still not significantly related in ribbon snakes

Figure 2-1. Positive relationship between median neonate SVL (NS) and median neonate mass (NM) in litters of garter snakes ($r^2=0.57$, $p<0.0001$, $n=67$) and ribbon snakes ($r^2=0.68$, $p<0.0001$, $n=41$).

Figure 2-2. Positive relationship between median neonate mass (NM) and maternal SVL (MS) in garter snakes ($r^2=0.19$, $p=0.0002$, $n=70$) and ribbon snakes ($r^2=0.14$, $p=0.01$, $n=42$).

Figure 2-3. Positive relationship between litter size (LS) and maternal size (MS) in garter snakes ($r^2=0.17$, $p=0.0004$, $n=70$) and ribbon snakes ($r^2=0.14$, $p=0.01$, $n=42$).

(multiple linear regression, $F_{2,39}=3.26$, $p=0.05$, $R^2=0.14$; maternal SVL: $F=6.15$, $p=0.02$; litter size: $F=2.25$, $p=0.14$).

Among-litter variation in offspring size

I predicted that among-litter variation in offspring mass should increase as litter size decreased. This relationship exists because the effect of energy not being evenly divisible by the optimal offspring size should be greater for smaller litters, where the addition of one more offspring represents a greater relative cost than in a larger litter. As was done by Weatherhead et al. (1999), I tested this prediction by calculating the coefficient of variation (CV) of median neonate mass among litters of equal size and regressed CV against litter size (for those litter sizes for which I had a minimum of four litters). The CV of neonate mass was not related to litter size in garter or ribbon snakes (GS: Pearson Product-Moment Correlation, $r=0.19$, $p=0.71$, $n=6$; RS: Pearson Product-Moment Correlation, $r=0.02$, $p=0.97$, $n=8$). Furthermore, I compared the observed variation in neonate size with the expected variation due to the fractional offspring hypothesis. In garter snakes, with a mean litter size of 12.8 and a mean of median neonate mass of 1.69 g, I expected neonate mass to range from 1.56 to 1.82 g. This corresponded to about 19% of the observed variation (within and among litters). In ribbon snakes, the expected range of neonate masses was from 1.26 to 1.58 g, because mean litter size was 9.0 and the mean of median neonate masses was 1.42 g. Thus, the fractional offspring hypothesis potentially explained approximately 32% of the observed variation (within and among litters). Although a greater amount of the observed variation in neonate size in garter and ribbon snakes might be attributed to the

fractional offspring hypothesis than in water snakes (Weatherhead et al. 1999), a large amount of variation still remained unexplained in my study species.

Within-litter variation in offspring size

The mean CVs for offspring mass and SVL within litters were 10.4% and 3.9% in garter snakes, and 7.5% and 3.4% in ribbon snakes. However, when I compared the within-litter variability in neonate size (SVL and mass) to maternal SVL and litter size, the only significant relationship was that neonate mass became more variable with maternal SVL in ribbon snakes (Table 2-1).

Effects of parturition date on offspring size

I predicted that neonate mass would increase as date of parturition increased because those neonates born later should benefit more from large size at birth. However, I found that median neonate mass decreased significantly with date of birth in garter snakes (Figure 2-4; linear regression, $F_{1,68}=5.09$, $p=0.03$, $r^2=0.07$) and was not related to date of birth in ribbon snakes (linear regression, $F_{1,40}=0.62$, $p=0.43$, $r^2=0.02$). I also tested for a negative relationship between litter size and date of birth, but found that they were not significantly correlated in garter or ribbon snakes (GS: Pearson Product-Moment Correlation, $r=-0.18$, $p=0.13$, $n=70$; RS: Pearson Product-Moment Correlation, $r=-0.17$, $p=0.28$, $n=42$). However, litter size was negatively correlated with relative date of birth in both species (Figure 2-5; GS: Pearson Product-Moment Correlation, $r=-0.27$, $p=0.02$, $n=70$; RS: Pearson Product-Moment Correlation, $r=-0.36$, $p=0.02$, $n=42$).

Table 2-1. Variability in offspring size measurements compared to maternal size and litter size. Neonate size was represented by the median for each litter, and the mean of those values is reported. Coefficients of variation (CV) were calculated for each litter, and the mean is presented. Comparisons were carried out as outlined in the text. Pearson product-moment correlation coefficients are presented, and significance is indicated (* $p<0.05$, ** $p<0.001$; *** $p<0.0001$).

	Garter Snakes			Ribbon Snakes		
	Correlation with		Litter size	Correlation with		Litter size
	Mean	Maternal SVL (cm)		Mean	Maternal SVL (cm)	
Offspring SVL (cm)						
Median	14.57	0.49***	-0.18	14.96	0.44*	-0.05
CV	3.9%	0.15	-0.12	3.4%	0.03	-0.09
Offspring mass (g)						
Median	1.69	0.44**	-0.04	1.42	0.31*	-0.09
CV	10.4%	0.007	-0.19	7.5%	0.32*	-0.09

Figure 2-4. Negative relationship of median neonate mass (NM) and parturition date (PD) in garter snakes ($r^2=0.07$, $p=0.03$, $n=70$).

Figure 2-5. Negative relationship between relative date of birth and litter size in garter snakes ($r=-0.27$, $p=0.02$, $n=70$) and ribbon snakes ($r=-0.36$, $p=0.02$, $n=42$). Relative date of birth was calculated as the number of days before or after the mean date of birth for that season.

Because offspring born earlier have more time to forage before entering hibernation, I predicted that they should allocate more energy into SVL than those neonates born later. When I controlled for differences in offspring mass, I found that median neonate SVL did not change with date of birth in garter or ribbon snakes (GS: multiple linear regression, $F_{2,64}=44.48$, $p<0.0001$, $R^2=0.58$; date of birth: $F=2.44$, $p=0.12$; median neonate mass: $F=88.85$, $p<0.0001$; RS: multiple linear regression, $F_{2,38}=44.06$, $p<0.0001$, $R^2=0.70$; date of birth: $F=2.53$, $p=0.12$; median neonate mass: $F=87.93$, $p<0.0001$). However, median neonate SVL, controlled for median neonate mass, increased with relative date of birth in garter snakes (multiple linear regression, $F_{2,64}=48.07$, $p<0.0001$, $R^2=0.60$; relative date of birth: $F=5.56$, $p=0.02$; median neonate mass: $F=96.04$, $p<0.0001$), but not in ribbon snakes (multiple linear regression, $F_{2,38}=40.61$, $p<0.0001$, $R^2=0.68$; relative date of birth: $F=0.31$, $p=0.58$; median neonate mass, $F=78.42$, $p<0.0001$).

Path analysis

I first analysed the effects of maternal size on litter size and of both variables on offspring size. As expected, maternal SVL and maternal mass were highly correlated in both garter and ribbon snakes (GS: Pearson Product-Moment Correlation, $r=0.84$, $p<0.0001$; RS: Pearson Product-Moment Correlation, $r=0.76$, $p<0.0001$). Holding all other factors constant, longer females gave birth to larger litters in garter snakes (Figure 2-6) and ribbon snakes (Figure 2-7). Heavier females also produced larger offspring in both species, but there was a trade-off between offspring size and offspring number within species. The overall effects of maternal SVL and maternal mass on neonate size were both positive in

Figure 2-6. Path diagram of relationships among reproductive variables in garter snakes. Non-causal correlation is indicated by a curved, double-headed arrow and represented by the Pearson product-moment correlation coefficient. Direct, causal effects are indicated by straight, one-way arrows and represented by standardised partial regression coefficients. Significant direct effects ($p < 0.05$) are indicated with a heavier line. Residual variables, indicated by U, represent the unexplained variance in the multiple regression for each dependent variable and were calculated as $(1-R^2)^{0.5}$.

Figure 2-7. Path diagram of relationships among reproductive variables in ribbon snakes. Coefficients were calculated as outlined for garter snakes (see Figure 2-6).

garter snakes (Table 2-2). However, in ribbon snakes, only maternal mass had an overall positive effect on neonate mass, whereas the relationship between maternal SVL and neonate mass was negative (Table 2-3).

In ribbon snakes, there was an unexpected negative relationship between maternal mass and litter size. It is possible that litter size decreased with maternal mass because mass was measured after parturition. A female with a larger litter might have invested a greater proportion of her resources into her current litter and kept fewer resources in reserve. Indeed, I found that the relative investment by ribbon snakes increased with litter size and decreased with body size (multiple linear regression, $F_{2,39}=106.27$, $p<0.0001$, $R^2=0.84$; litter size: $F=204.32$, $p<0.0001$; maternal mass: $F=28.57$, $p<0.0001$). Similarly, in garter snakes, RCM increased as litter size increased, and decreased with maternal mass (multiple linear regression, $F_{2,67}=82.10$, $p<0.0001$, $R^2=0.71$; litter size: $F=158.66$, $p<0.0001$; maternal mass: $F=44.62$, $p<0.0001$). However, when I divided a female's RCM by the number of offspring in her litter, I found that the relative effort per offspring by a ribbon snake (3.3%) was significantly higher than that of a garter snake (2.6%; t-test, $t=5.045$, $p<0.0001$). Because neonates born later in the season had less time to forage before entering hibernation, I predicted that they would be born larger. However, neonate mass decreased as date of parturition increased in garter snakes (Table 2-2) and only increased slightly with an increase in parturition date in ribbon snakes (Table 2-3). Controlling for differences in neonate mass, I found that neonate SVL decreased as date of parturition increased.

Table 2-2. Overall effect coefficients (sum of direct and indirect effects from path analysis) between reproductive variables in garter snakes, *Thamnophis sirtalis*.

	Effect on			
	Litter size	Parturition date	Time in captivity	Neonate mass
Maternal SVL	0.40	-0.13	-0.04	0.29
Maternal mass	0.02	-0.02	-0.007	0.17
Capture date		0.09	-0.90	0.07
Litter size		-0.15	-0.05	-0.30
Parturition date			0.34	-0.24
Time in captivity				-0.10
Neonate mass				0.80

Table 2-3. Overall effect coefficients (sum of direct and indirect effects from path analysis) between reproductive variables in

ribbon snakes, *Thamnophis sauritus*

	Effect on				
	Litter size	Parturition date	Time in captivity	Neonate mass	Neonate SVL
Maternal SVL	0.60	-0.14	-0.04	-0.20	-0.13
Maternal mass	-0.28	-0.01	-0.003	0.61	0.51
Capture date		0.33	-0.96	0.31	0.24
Litter size		-0.19	-0.05	-0.19	-0.13
Parturition date			0.28	0.09	-0.08
Time in captivity				-0.27	-0.26
Neonate mass					0.82

Although this pattern occurred in the expected direction, the overall effect coefficients were quite small. Interestingly, I also found that larger litters were born earlier than smaller litters.

Lastly, I investigated the effects of an increase in a female's time in captivity on the characteristics of her offspring. In garter snakes, I found that an increase in time in captivity led to a slight decrease in neonate mass and an increase in neonate SVL. However, in ribbon snakes, the effect of captivity on neonate mass and neonate SVL was negative. The overall effects of captivity on garter snake neonate size were negligible (Table 2-2). In ribbon snakes, an increase in the time that a female was held in captivity corresponded to a decrease in the size of the neonates in her litter (Table 2-3).

Using relative date of birth in the path analysis, I found that most of the relationships remained the same. However, the effect of capture date on parturition date decreased greatly in ribbon snakes, and became negative in garter snakes. In ribbon snakes, the direct effect of litter size on parturition date became significantly negative and the overall effect of parturition date on neonate mass was negative.

Indices of offspring quality

When I compared the indices of offspring quality in all neonates, I found that longer neonates had more ventral scales and swam faster than shorter neonates in both garter and ribbon snakes (Table 2-4). However, swimming speed increased as the number of ventral scales increased in garter snakes only. When I compared these measures of offspring quality to maternal SVL and litter size, I found inconsistent trends (Table 2-5). Swimming speed was not related to maternal SVL or litter size in garter or ribbon snakes. In garter snakes,

Table 2-4. Correlations of indices of offspring quality in garter snakes and ribbon snakes. Indices were compared with

Spearman rank correlations (ρ), and significance and sample size are reported.

Comparison	Garter Snakes			Ribbon Snakes		
	ρ	p-value	Sample size	ρ	p-value	Sample size
SVL - Ventral scales	0.36	<0.0001	744	0.46	<0.0001	353
SVL - Swimming speed	-0.22	<0.0001	601	-0.32	<0.0001	320
Ventral scales - Swimming speed	-0.15	0.0002	600	-0.04	0.46	315

Table 2-5. Relationship of offspring quality indices (number of ventral scales and swimming speed) to maternal size and litter size. Results of a multiple linear regression of each quality index against maternal SVL and litter size are presented.

Index	Variable	Garter Snakes				Ribbon Snakes			
		F	df	p	R ²	F	df	p	R ²
Ventral Scales	Overall analysis	2.46	2,63	0.09	0.07	1.24	2,38	0.30	0.06
	Maternal SVL	4.84		0.03		0.05		0.82	
	Litter size	1.16		0.29		2.29		0.14	
Swimming Speed	Overall analysis	0.20	2,54	0.82	0.007	0.73	2,36	0.49	0.04
	Maternal SVL	0.04		0.85		0.88		0.35	
	Litter size	0.39		0.53		0.08		0.78	

there was weak evidence for a positive effect of maternal SVL on the median number of ventral scales in neonates.

Discussion

Although Smith and Fretwell's (1974) optimal offspring size theory is extensively cited in the literature, variation in offspring size has been observed across many taxa (Roff 1992, Stearns 1992, Bernardo 1996a). In snakes, the applicability of the concept of an optimally-sized offspring in a given population has been questioned because offspring size varies greatly within and among litters (Weatherhead et al. 1999). My main goal in this study was to investigate the importance of various factors in the observed variation in offspring size, and to compare these observations to those from previous studies of snakes. First, in univariate analyses, neonate size increased significantly with maternal SVL in both garter and ribbon snakes. Litter size also increased with maternal SVL, and there was some evidence of a trade-off between offspring size and number. Consequently, it is likely that the indirect effect of maternal size on neonate size, through litter size, might offset the direct, positive effect. In the path analysis, I found that the overall effect of maternal SVL on neonate mass was positive in garter snakes and negative in ribbon snakes.

In garter snakes, the direct effect of maternal mass on litter size was negligible, while maternal and neonate mass were positively related. In ribbon snakes, the relationship between maternal mass and litter size in ribbon snakes was negative, and the positive, direct effect of maternal mass on neonate mass was significant. Overall, the effects of maternal mass on neonate mass were positive in both study species. The negative relationship

between maternal mass and litter size in ribbon snakes was unexpected. I have suggested that this relationship might have been found because the measurement of maternal mass was taken after parturition. Indeed, I found that females invested a greater proportion of their resources into larger litters, likely leaving them with fewer resources in reserve. Thus, females that produced large litters may have weighed less after parturition. Furthermore, I found that the relative investment per offspring was greater in ribbon snakes than garter snakes. Therefore, the production of a larger number of offspring would have caused a ribbon snake female to invest a greater proportion of her energy into her young rather than keeping it in reserve. Additionally, if ribbon snake females were able to supplement their embryos during development, as shown in some viviparous snakes (Clutton-Brock 1991), larger females should produce larger offspring. Thus, the large overall effect of maternal mass on neonate size in ribbon snakes might have resulted from placental nourishment, which was less important in garter snakes.

Although the trade-off between offspring size and number is consistent with optimal offspring size theory when it occurs at the among-population level, the observation of such a relationship within populations is less well understood. None of the existing hypotheses seemed to explain the observed trade-off in water snakes (Weatherhead et al. 1999). However, in garter and ribbon snakes, I found that females invested a greater proportion of their resources into larger litters, as assumed in Winkler and Wallin's (1987) model. In this model, optimal offspring size was shown to vary with litter size. Because litter size varied with maternal size in my study species, it is not surprising that offspring size also varied with

maternal size. However, the value of referring to an optimal offspring size that changes with litter size and maternal size is debatable.

I predicted that females that gave birth later should produce larger offspring. However, I found that neonate mass decreased with parturition date in garter snakes, and increased only slightly with parturition date in ribbon snakes. As in two previous studies of snakes, I also found that larger litters were born earlier (King 1993, Weatherhead et al. 1999). As outlined previously, this might have occurred because larger females, who produced larger litters, had greater thermal inertia or ovulated earlier in the season. Because I found that maternal mass and litter size were negatively related in ribbon snakes, the "thermal inertia" hypothesis is less likely to explain this pattern than the "timing of ovulation" hypothesis. The latter explanation was also favoured by Weatherhead et al. (1999) because it was more consistent with their data from captive and free-living water snakes. I also expected to find that neonate SVL decreased with parturition date because those neonates born later should hold more of their energy in reserve. In my univariate analysis, I found that neonate SVL increased with parturition date in garter snakes, and was not related to parturition date in ribbon snakes. However, I found a negative, albeit weak, relationship between date of parturition and neonate SVL in both study species in the path analysis.

Lastly, I investigated the effects that a female's time in captivity had on the size of her offspring. The direct effects of time in captivity on neonate mass were negative in both study species. The overall effects on neonate mass and neonate SVL were quite small in garter snakes. However, in ribbon snakes, neonate mass and neonate SVL decreased

considerably with an increase in time in captivity. Thus, the effects of captivity were more severe in ribbon snakes than in garter snakes. As suggested previously, ribbon snakes might be candidates for placental nourishment. If they are able to supply their embryos with resources during development, it is not surprising that the stressful conditions of captivity might have greater effects on their young.

As in a study of *Thamnophis marcianus* (Seigel and Ford 1992), I found that within-litter variance in offspring size (SVL or mass) was not significantly related to maternal size or litter size in both garter and ribbon snakes. This means that the variability of offspring in a litter did not depend on the size of their mother or on the number of offspring in the litter. However, in the previous study, within-litter variation in offspring SVL was higher in litters of females on a high-energy diet than in litters of females on a low-energy diet (Seigel and Ford 1992). A similar study could be conducted in garter and ribbon snakes to determine whether females allocated their resources into more variable offspring when environmental conditions were not stressful (i.e. high-energy diet).

In the last part of this study, I compared other indices of offspring quality in both study species. The number of ventral scales, which represented the number of vertebrae, increased with neonate SVL in garter and ribbon snakes. In both study species, longer neonates also swam more quickly than shorter neonates. This suggests that those females with enough resources to invest in larger offspring also produced better quality offspring. Thus, the higher survival rate of longer neonates observed in previous studies (i.e., Jayne and Bennett 1990) might have resulted simply from larger size (and more energy), or from superior locomotory performance, which allowed them to escape predators successfully.

However, I found no evidence that offspring size was sacrificed for an increase in other measures of offspring quality. Thus, variation in offspring quality can not be used to explain the observed variation in offspring size.

Because the trade-off between offspring size and number is very common in snakes (King 1993, Madsen and Shine, Weatherhead et al. 1999, present study), it is not surprising that offspring size and litter size both vary within populations. Furthermore, offspring size has been found to vary with maternal size in both viviparous and oviparous snakes (Ford and Killebrew 1983, Ford and Karges 1987, Seigel and Ford 1991, King 1993, Madsen and Shine 1996, present study). Even in northern water snakes, where no simple relationship was found between maternal and offspring size, the direct effect of maternal size (controlling for litter size) on offspring size was positive (Weatherhead et al. 1999). Thus, the dependence of offspring size on maternal size, litter size and parturition date that I have shown in the present study demonstrates that numerous environmental and maternal factors influence variation in offspring size, and no single, optimal offspring size can be identified for many species.

A great deal of variation in offspring size remained unexplained in my path analysis, as indicated by the high residual variables. In a future study, a new path diagram should be constructed and used to test the effect of each variable on offspring size. In my path analysis, I demonstrated the importance of factors such as maternal size and litter size. Thus, future studies should concentrate on their effects on offspring size, as well as considering the magnitude of the effects of other variables. For example, the feeding behaviour and growth rate of a female during gestation might contribute to the size of her offspring,

especially if she is able to feed her embryos by placental nourishment. Additionally, the condition of the female at ovulation or throughout gestation could also play a role in determining the amount of energy that a female invests into her litter and how she allocates that energy to her offspring. However, the new model should remove those variables, like time in captivity, that do not contribute significantly to variation in offspring size.

Overall, I have shown that many of the patterns observed in previous studies also exist in garter and ribbon snakes. First, larger females do produce larger litters, as in numerous snake species (Ford and Killebrew 1983, Ford and Karges 1987, Brodie and Ducey 1989, Seigel and Ford 1991, Larsen et al. 1993, King 1993, Madsen and Shine 1996, Greene et al. 1999, Weatherhead et al. 1999). I also found that these larger litters were born earlier, in accordance with the findings in brown snakes and water snakes (King 1993, Weatherhead et al. 1999). Because larger neonates have a better chance of survival (Jayne and Bennett 1990), females should maximise their own fitness by producing larger neonates. They can do this by giving birth early in the season to small neonates that have sufficient time to forage before entering hibernation. Alternatively, females that are not able to accelerate the timing of parturition should give birth to larger neonates later in the season. Females that give birth earlier likely improve their own chances of survival because they have more time to replace energy invested in their litter before entering hibernation (Brown and Weatherhead 1997, Greene et al. 1999). Therefore, as suggested by Weatherhead et al. (1999), females should manipulate the trade-off between offspring size and number in relation to their body size, and thus, their ability to control timing of parturition. Indeed, in the three studies that have investigated timing of parturition and the relationship between

offspring size and number, larger litters of smaller offspring were born earlier (King 1993, Weatherhead et al. 1999, present study). However, there is still a need for studies that compare the survival rates of neonates among litters to establish whether the trade-offs between offspring number, size and quality are manipulated by females adaptively.

SUMMARY

1. In garter snakes, *Thamnophis sirtalis sirtalis*, and ribbon snakes, *Thamnophis sauritus septentrionalis*, females were longer and heavier than males as adults. Sexual size dimorphism did not occur in the expected direction in neonates of either study species. In fact, female ribbon snake neonates were significantly smaller than males.
2. Ribbon snake litters containing larger neonates were more male-biased, but maternal size and sex ratio were not related. Furthermore, sex ratio was not related to maternal size in garter or ribbon snakes.
3. A high number of stillbirths occurred during this study. However, neither the presence nor the number of stillborn young in a litter depended on maternal or litter size. Similarly, an increase in the length of time that a female was held in captivity did not increase the number of stillborn young in her litter.
4. Larger females gave birth to larger litters of offspring. There was a direct, positive relationship between maternal size and neonate size, as well as a trade-off between offspring size and number.
5. In both study species, larger litters were born earlier than smaller litters, but date of parturition did not seem to affect offspring size.
6. An increase in the length of time that a female was held in captivity led to a substantial decrease in offspring size in ribbon snakes.
7. Indices of offspring quality (number of vertebrae and swimming performance) were positively correlated with offspring size. Thus, garter and ribbon snakes do not seem to trade off offspring size and quality.

8. Patterns of variation in offspring size or litter sex ratios are inconsistent among snake species. Additional studies are required to elucidate the extent of maternal control over offspring fitness.

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