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**MATERNAL INFLUENCES ON OFFSPRING QUALITY IN
NORTHERN WATER SNAKES, *NERODIA SIPEDON***

by

Kelley J. Kissner

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

Doctor of Philosophy

**Carleton University
Ottawa, Ontario**

7 January 2002

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“MATERNAL INFLUENCES ON OFFSPRING QUALITY
IN NORTHERN WATER SNAKES, *NERODIA SIPEDON*”

submitted by KELLEY J. KISSNER, B.SC., M.SC.
in partial fulfillment of the requirements for
the degree of Doctor of Philosophy

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ABSTRACT

A female's fitness is intimately related to the survival and reproductive success of her offspring (Roff 1992). Thus, natural selection should have shaped female reproductive strategies in ways that maximise the collective fitness of a female's offspring, and thus maximise the female's own fitness. Here I explore the ability of female northern water snakes (*Nerodia sipedon*) to enhance their fitness by increasing the quality of their offspring through their mating behaviour and through maternal effects on offspring phenotypes.

At a given litter size, larger female water snakes invested in larger offspring and females that produced larger litters produced smaller offspring. Variation in offspring size among females' litters appears to be a result of proximate factors limiting females' ability to invest in their offspring. An experiment that regulated females' ability to thermoregulate during gestation revealed that basking opportunity influenced several phenotypes of offspring including size, condition, and locomotory performance. However, providing females with unlimited opportunity to maintain their preferred temperature during gestation did not produce offspring that were optimal in all phenotypes examined. In addition, free-living females and females provided with basking opportunities similar to those available in the wild did not produce offspring with superior phenotypes. These results suggest that individual phenotypes are sensitive to differences in temperature and, consequently, no overall temperature can optimise all phenotypes simultaneously. However, these results do not preclude the possibility that females can optimise some traits of their offspring at the expense of others.

Larger males had higher reproductive success than smaller males. Offspring sired by larger males had proportionally higher survival over winter in hibernation sites constructed in outdoor enclosures. Thus, females can acquire 'good genes' for their offspring by mating with large males. Multiply sired litters were common (54% of litters) and survival of offspring from multiply sired litters was higher. Thus, females receive indirect fitness benefits by engaging in multiple mating. The enhanced survival of offspring in multiply-sired litters may be due to increased phenotypic diversity of litters or to genetic benefits.

ACKNOWLEDGEMENTS

As I write these acknowledgements, I am struck by how much of a group effort this project was and how many people I have to thank. To all of you cited below, please recognize that these words do not adequately describe the depth of my gratitude.

First and foremost, I thank my supervisor, Dr. Patrick Weatherhead, for his guidance and support, and for providing a productive and enjoyable learning environment. Pat, thank you for sharing your incredible knowledge and understanding of evolution and animal behaviour with me. I have learned so much. Your commitment to science has motivated me to follow higher standards in my research, and I have benefited greatly from being part of your lab. I am particularly grateful for your time, patience, encouragement, and guidance during the writing process. Thank you for reading and commenting on my work and for making communication over a long distance easier than expected.

I thank my advisors, Dr. Frances Pick and Dr. Myron Smith for their advice, guidance, and constructive criticism. I also thank Dr. Smith and his students for allowing me to do DNA extractions in their lab. In addition, I thank Dr. Mark Forbes for his support, guidance, and friendship throughout my entire experience as a graduate student. Mark, you have always been source of advice and encouragement for me. I truly appreciate the amount of time you have given me.

I thank Dr. Lisle Gibbs for bringing me into his lab to conduct my genetic analysis. I thank Dr. Gibbs and the technicians and students in the Gibbs' lab for teaching me about microsatellite techniques and paternity analysis and for encouraging

me through this part of my study. I am extremely grateful to L. DeSousa, B. Woolfenden, M. Kasumovic, and L. Blanchard for running PCRs and gels for me.

I thank Queen's University for allowing me to conduct research at Queen's University Biology Station and for allowing me to alter a section of Hughson Tract to construct the snake enclosures. I thank the staff at QUBS, particularly F. Phelan, M. Phelan, F. Connor, and R. Green, and the other researchers at QUBS for providing an enjoyable environment in which to live and work.

Financial support for this project was provided by grants from the Natural Sciences and Engineering Research Council of Canada (NSERC) to Patrick J. Weatherhead and H. Lisle Gibbs. Personal financial support came from scholarships from NSERC, the province of Ontario, and Carleton University. Additional support for myself was provided by Dr. Weatherhead's research grants from NSERC and University of Illinois.

This project would not have been possible if it were not for all who helped to construct and maintain the snake enclosures. I thank P. Weatherhead for his design of the enclosures and hibernation sites and G. Blouin-Demers for additional advice during construction. For digging, planting and trimming vegetation, catching minnows, etc., I thank P. Weatherhead, G. Blouin-Demers, G. Brown, E. O'Grady, M. Prosser, C. Verrault, F. Phelan, F. Connor, R. Green, S. Sommerer, J. Gilchrist, D. Bender, K. Metz, J. Svec, H. McCracken, T. Volk, A. Moenting, C. Yourth, P. Begin, S. Doucet and J. Jones. I am sorry for all the sore muscles and scrapes you had to put up with!

For catching and caring for snakes, for assistance with other work in the field, and for keeping me laughing during my field seasons, I owe a huge amount of gratitude to G.

Brown, S. Sommerer, J. Gilchrist, J. Svec, H. McCracken, T. Volk, A. Moenting, E. O'Grady, K. Cavey, P. Begin, and S. Doucet.

I thank my lab mates and friends who provided support, encouragement, statistical advice, editorial comments, and recreation. Thank you G. Brown, S. Sommerer, J. Svec, H. McCracken, G. Blouin-Demers, K. Dufour, M. Prosser, K. Prior, M. Gunness, D. Bender, D. McCurdy, B. Leung, L. DeSousa, D. McRuer, C. Chabot, B. Gummer, W. Gummer. Three people deserve special mention for their commitment to me and this project. Greg Brown, for teaching me so much of what I know about water snake biology, for providing assistance and information whenever required, and, most of all, for his unwavering support and friendship. Sophie Sommerer, for being an amazing field assistant, colleague, and friend. Darren Bender for supporting me during all my graduate research, for his willingness to help at a moment's notice, and for his enduring friendship and faith in me, all of which greatly contributed to the completion of this thesis.

Finally, I thank my family for their love and incredible support. I particularly thank my sister, Krista, and brother-in-law, David, for bringing me into their home while I wrote my thesis and for their love, encouragement, and understanding during the process. I thank their new addition to our family, Kaitlyn, for keeping me smiling during my final months of writing. I thank my parents, Don and Marjorie, for their love and encouragement, especially during the final phase of this project. I also thank my grandmother, Gladys Tippet, for always encouraging and supporting my studies.

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GENERAL INTRODUCTION

A female's fitness is intimately related to the survival and reproductive success of her offspring (Roff 1992). Thus, natural selection should have shaped female reproductive strategies in ways that maximise the collective fitness of a female's offspring, and thus maximise the female's own fitness. There are two general ways that a female can influence the quality of her offspring. First, a female may control who she mates with and/or her number of mates. If males differ in quality in ways that are identifiable to females, females should preferentially mate with only high quality males. Although the idea of females choosing among mates was initially contentious, there is abundant evidence that females discriminate among males and that these decisions can affect the quality of offspring. For example, peahens (*Pavo cristatus*) that mate with peacocks with more elaborate trains give birth to offspring with higher growth and survival rates (Petrie 1994). Similarly, female house mice (*Mus musculus*) provided with choice over their mates have offspring with higher viability than females provided with no choice (Drickamer et al. 2000).

If females cannot discriminate against males prior to mating, or if females have limited control over with which males they mate, females may enhance the quality of their offspring by mating with several males. Multiple mating is common in a variety of taxa (e.g., birds: Gladstone 1979; Gibbs et al. 1990; mammals: Hanken and Sherman 1981; Davies and Boersma 1984; Murie 1995; insects: Ochoa et al. 1996; Tregenza and Wedell 1998; reptiles: Barry et al. 1992; Madsen et al. 1992; Olsson et al. 1994a,b, 1996a,b). Because mating with several partners involves costs such as time, energy, and predation risk, much debate and research has focussed on determining how females

benefit from multiple mating (Schwagmeyer 1984; Halliday and Arnold 1987). Multiple mating may provide insurance against a male being sterile (Gibson and Jewell 1982) or sperm limited (Gromko et al. 1984). Multiple mating also may allow females to exercise post-copulatory choice of mates (Olsson et al. 1994a,b, 1996a,b; Eberhard 1996) or promote intrauterine sperm competition whereby ova are fertilized by the most competitive sperm and thus by the genetically superior male (Curtsinger 1991). Finally, females may increase the genetic heterogeneity of their litters, increasing the possibility that some offspring will survive (Williams 1975). Empirical studies support the hypothesis that females can receive indirect fitness benefits from mating with multiple males. For example, female adders (*Vipera berus*) and sand lizards (*Lacerta agilis*) that mate with multiple males produce more viable offspring (Madsen et al. 1992; Olsson et al. 1994b, 1996a).

The second general way that females can enhance the quality of their offspring involves varying the phenotypes of their offspring. There is now abundant empirical evidence that the phenotype, behaviour, and environment of females can have dramatic influences on the phenotypes of their offspring (Bernardo 1996a,b; Mousseau and Fox 1998). These types of influences on offspring phenotypes that are unrelated to offspring genotypes, have generally been referred to as 'maternal effects' (Bernardo 1996a). Where once maternal effects on offspring phenotypes were ignored or statistically removed, the recognition of the potential for maternal effects to be adaptive has increased their interest to evolutionary biologists (Bernardo 1996a,b; Mousseau and Fox 1998).

The most widely recognized and studied maternal effect is that on offspring size. Females must make "decisions" involving the trade off between the number and size of

offspring they produce. Offspring size commonly varies among females within populations, contrary to the theoretical expectation that offspring size should not vary (Bernardo 1996a,b; Weatherhead et al. 1999). Evidence that maternal effects account for variation in offspring size are correlations between the traits or circumstances of females and the size of their offspring (Bernardo 1996a,b).

Maternal effects also act on traits other than offspring size. For example, the environment in which females rear their offspring can also influence the phenotypes of their offspring. This is particularly true of ectothermic animals in which development of eggs or embryos is directly influenced by environmental conditions. For example, in species that lay eggs, the environment in which females choose to oviposit may influence the size, shape, behaviour, and even the sex of offspring (Bull 1980; Burger 1989, 1990, 1998a,b; Shine et al. 1997a,b). In species that carry their embryos throughout gestation, the environmental conditions embryos experience during development are mediated by the behaviour of the mother (e.g., via thermoregulatory behaviour) and can affect offspring phenotypes (Sorci and Clobert 1997; Wapstra 2000).

The goal of my thesis is to investigate the potential for female northern water snakes (*Nerodia sipedon*) to influence the quality of their offspring through their mating behaviour and through maternal effects on offspring phenotypes. I then determine the fitness consequences for females exhibiting these behaviours by measuring over-winter survival of offspring in hibernation sites constructed in outdoor enclosures.

Snakes are well suited to this investigation because there are only a limited number of ways in which females can influence the quality of their offspring. Female snakes do not provide maternal care for young once they are born, so maternal

investment in offspring is complete at birth. In addition, male snakes do not provide paternal care for offspring so their contribution is restricted to their genes. Water snakes are particularly well suited to this study because they are viviparous and thus, factors such as temperature that may affect development of offspring are mediated through the mother. Furthermore, the reproductive cycle and embryogenesis of northern water snakes are relatively well studied.

In Chapter One, I investigate factors influencing reproductive success of males and multiple mating by females. I use seven hypervariable microsatellite DNA loci to assign paternity to neonates to determine parentage of litters. To determine the factors influencing male reproductive success, I examine whether males that are larger, in better condition, have longer tails, or that are less genetically related to females have higher reproductive success. I also examine whether the operational sex ratio (OSR), defined as the number of reproductively active males to females, influences whether phenotypes of males are important in determining success. I hypothesize that when the OSR is male-biased and competition among males is more intense, male phenotypes may be more important in determining success. To examine factors influencing multiple mating by females, I examine whether phenotypes of females influence their likelihood of producing multiply sired litters. I test the hypothesis that females who produce larger litters may be more likely to mate with multiple males if larger litter size makes them more attractive to males. Finally, I examine several adaptive hypotheses of why females engage in multiple matings, including: to insure against a male being sperm-limited, to decrease the possibility of producing offspring with related males, and to increase the genetic diversity of their litters.

My first goal in Chapter Two is to investigate maternal effects on offspring size. Specifically, I consider how maternal size, condition, litter size, and food intake influence female investment into the size of their offspring and litters. I also consider how consistent individual females are in the size of offspring they produce across their litters to investigate whether ecological or genetic differences among females are relatively more important in determining the size of offspring that females produce. I predict that low consistency would indicate that ecological factors account for variation in offspring size and high consistency would suggest that genetic factors are more important. My second goal in this chapter is to address maternal effects on sex ratio variation. Water snakes are sexually dimorphic in size, with females being the larger sex. Thus, any factor that gives offspring a head start on growth should result in litters being disproportionately female (Weatherhead et al. 1999). Thus, I examine whether females who produce larger offspring and give birth earlier have litters that are disproportionately female. I also examine whether the expression of tail length, a sexually dimorphic trait in water snakes, is dependent on the sex ratio of offspring *in utero*. During development, male and female offspring are exposed to circulating sex hormones of the opposite sex. Thus, I test the hypothesis that tail length of females should increase (i.e., become masculinized) with an increasing proportion of males in litters and tail length of males should decrease (i.e., become feminized) with an increasing proportion of females in litters.

In Chapter Three, I address a second maternal effect, that of the maternal thermal environment on phenotypes and behaviour of offspring. I provide captive females with varying opportunity to bask during gestation to test two alternative hypotheses. First, the greater the opportunity females have to maintain their body temperatures in their

preferred range, the more likely it is that offspring will have characteristics that should be favourable for survival (i.e., “warmer is better”). Second, the more closely thermoregulatory opportunities mimic patterns experienced by females in the wild, the more likely it is that offspring will have characteristics that should be favourable for survival (i.e., “natural is better”). I also examine whether any effects of maternal basking opportunity on offspring phenotypes persist, change, or emerge through time by comparing phenotypes and behaviour of offspring measured at birth with those measured a second time just prior to hibernation.

In Chapter Four, I conduct an experiment on over-winter survival of offspring hibernated in outdoor artificial hibernation sites to determine whether fitness of females varies in relation to patterns found in Chapters One to Three. Specifically, I examine which phenotypes influence over-winter survival of offspring. I also examine whether females acquire ‘good genes’ for their offspring by mating with males with particular phenotypes and whether females that mate with multiple males receive indirect fitness benefits via increased survival of their offspring.

CHAPTER ONE
MATING BEHAVIOUR OF FEMALE NORTHERN WATER SNAKES,
NERODIA SIPEDON

Introduction

A cornerstone of Darwin's (1871) sexual selection theory was the proposition that females actively select their mates from the available males. The idea that females could choose among potential mating partners was initially contentious, but overwhelming empirical evidence supports Darwin's suggestion (reviewed by Pomiankowski 1988; Andersson 1994; Jennions and Petrie 1997). Reproductive success of females is dependent on the quality of their offspring because females generally cannot increase their number of offspring by mating with several males. Thus, females can enhance their fitness by preferentially mating with males that provide superior parental care to their offspring, or those males that are of higher genetic quality, and will pass that quality on to their offspring. Because paternal care is absent in snakes, female snakes can only increase their fitness by preferentially mating with high quality males.

There are two ways in which females could increase the possibility that their offspring are sired by high quality males. First, as Darwin (1871) suggested, females could choose among potential mates. Empirical evidence supports the idea that females can acquire 'good genes' for their offspring by being selective about the males with which they mate (Norris 1993; Moller 1994; Petrie 1994). Second, females could mate with multiple males. Multiple mating may result in increased offspring viability as a result of females choosing among sperm (Eberhard 1996; Olsson et al. 1996b). Alternatively, multiple mating may promote intrauterine sperm competition whereby ova

are fertilized by the most competitive sperm, and thus presumably by the genetically superior male (Curtisinger 1991; Madsen et al. 1992). Finally, females who produce offspring with several males should enhance the genetic diversity of their litters to increase the probability that some offspring will survive (Williams 1975).

Modern genetic tools have revolutionized studies of reproductive behaviour. These tools allow for the accurate assignment of paternity of offspring and are important because behavioural observations of mating success are often unreliable indicators of true mating success (e.g., Westneat et al. 1987; Gibbs et al. 1990; Prosser 1999). In addition, a comprehensive understanding of mating behaviours, such as multiple mating, requires that the number of sires in each litter and the number of offspring sired by each male are known. In this chapter, I used DNA-based analysis of paternity of offspring to examine factors associated with reproductive success of males and multiple mating by female northern water snakes (*Nerodia sipedon*).

Water snakes are well suited to this type of study. Water snakes mate in aggregations consisting of one female and one to several males (Tinkle and Liner 1955). Males attempt to entwine their tails with that of a female in order to insert their hemipenes and attempt to dislodge other males from the female (Mushinsky 1979). Male snakes cannot forcibly copulate with females because they cannot force apart the scales covering a female's vent (Devine 1984). Consequently, females apparently have some choice over the males with which they mate. In addition, females often mate with several males in a single breeding season, resulting in 54–86% of litters being sired by multiple males (Barry et al. 1992; Weatherhead et al. 1995; Prosser 1999). Thus, this species is appropriate for investigating factors influencing multiple mating by females.

Unlike other taxa, such as birds, there is little information on mate choice in snakes. Male snakes lack conspicuous secondary sexual traits that might serve as cues to quality. However, males do vary in traits such as body size and condition that could provide some information on male quality. Males of many snake species compete for females in ritualized combat between two males, or in scramble competitions involving large numbers of males. In several species, males that are larger, in better condition, or have longer tails have higher mating success (Shine 1978; Madsen and Shine 1993; Madsen et al. 1993; Weatherhead et al. 1995; Luiselli et al. 1996; Shine et al. 2000). While it is not known whether male success varies due to females actively choosing to mate with males with these characteristics or passively mating with the winners of mating competitions, functionally it probably does not matter. Passive mate choice (accepting the winner of competitions among males) and active mate choice can have the same fitness consequences for females. Females who actively choose to mate with males based on a phenotypic trait presumably do so because this trait reflects male quality. Similarly, males who possess a trait that makes them competitively superior in mating competitions presumably are of higher quality than males that lose competitions.

Recently, Prosser (1999) showed that the variance in reproductive success in water snakes was higher in males than females, resulting in the opportunity for sexual selection to act on male traits being approximately five times higher in males than in females. Despite variation in reproductive success among males, however, Prosser (1999) failed to find that body size, condition or tail length influenced male reproductive success. However, a previous study of mating patterns in water snakes showed that successful males were larger in one of two years, but did not differ from unsuccessful

males in body condition or relative tail length (Weatherhead et al. 1995). In taxonomically related species such as garter snakes (*Thamnophis* spp.) and grass snakes (*Natrix* spp.), males that are larger, in better condition, and/or have longer tails are more successful (Madsen and Shine 1993; Shine et al. 1999, 2000; but see Joy and Crews 1988). Because existing evidence for water snakes is contradictory, my first objective in this chapter is to determine whether attributes of males influence their mating success. I also investigate whether the number of males competing for females influences whether male attributes are important in determining male success. I test the prediction that when the operational sex ratio (OSR), defined as the ratio of reproductively active males to fertilizable females, is male-biased, male attributes will be more important in determining success of males because competition among males should be high, whereas when the OSR is female-biased, male attributes will be less important. My second objective is to examine whether the proportion of offspring sired in multiply sired litters varies with the size, condition, or tail length of the males that sired those offspring. I hypothesized that a male's success should be proportional to the amount of sperm provided to the female. In turn, the amount of sperm should be influenced by male size, because male size and testis size are correlated (Shine et al. 1999). Similarly, males in better condition should be more successful if better condition allows those males to produce more sperm or replenish sperm more quickly. Finally, males with proportionately longer tails may have a mating advantage if longer tails allow males more effective intromission with females (King 1989). Thus, I predicted that males that are larger, in better condition, and have longer tails relative to their competitors would sire proportionally more offspring.

Another way in which body size could affect the outcome of mating competitions could be the occurrence of size-assortative mating, whereby large males tend to mate with large females, and small males with small females. If sperm is costly to produce, if sperm competition is intense, or if the quality of females is variable, males should be selective about the females with which they attempt to mate (Sargent et al. 1986; Schwagmeyer and Parker 1990; Olsson 1993; Shine et al. 2001). Larger females might be more attractive to males due to their higher fecundity. If larger males are more competitive in mating aggregations, they should mate with larger females (Prosser 1999). Size-assortative mating has been documented in water snakes (Prosser 1999) and in other species of snakes (e.g., Shine et al. 2001) and lizards (Olsson 1993; Cooper and Vitt 1997). My third objective is to investigate the influence of variation in the OSR on assortative mating in water snakes by varying sex ratios of females and males housed together. Highly male-biased OSR should result in only the most competitive males siring offspring. If large males are more competitive, large males should monopolize large females, forcing smaller males to mate with small females. At female-biased OSR, competition among males should be lower, resulting in small and large males having the opportunity to mate with large females. Thus, I predicted that males would be more likely to mate assortatively under highly male-biased OSR.

A recent study of an inbred population of sand lizards (*Lacerta agilis*) demonstrated that the proportion of offspring sired by each male in multiply sired litters was biased in favour of males genetically most different from the female, despite females mating with almost every male that courts them (Olsson et al. 1996b). Because inbreeding may result in decreased offspring viability (Charlesworth and Charlesworth

1987; Madsen et al. 1996) selection should favour females (or eggs) that can discriminate against the sperm of related males (Olsson et al. 1996b). While not inbred, marsh populations of water snakes used in this study have restricted gene flow, possibly due to low dispersal from natal marshes (Prosser et al. 1999). Thus, there is some possibility for mating to occur between close relatives. Prosser (1999) found no evidence that the degree of relatedness among male and female water snakes determined which snakes produced offspring together. However, Prosser (1999) did not examine how paternity within multiply sired litters was influenced by the degree of relatedness. If females are unable to discriminate against related males prior to copulation, it is possible that discrimination occurs after mating. Under these conditions, only females that mate with multiple males would have the opportunity to discriminate against related males. Consequently, my fourth objective is to examine whether females producing litters sired by multiple males bias paternity of offspring in favour of the least genetically related male (Olsson et al. 1996b).

Multiple mating by females may be another way females can influence the quality of their young, particularly if cues about male quality are absent or unreliable (Olsson and Madsen 1995). Barry et al. (1992) found that the incidence of multiple paternity in water snakes was 86% during one mating season. A more recent study conducted over three mating seasons showed that 54% of female water snakes produce litters sired by multiple males (Prosser 1999). My fifth objective is to determine which factors are associated with multiple mating by female water snakes. Prosser (1999) proposed that if males prefer to mate with larger females, multiple mating might be influenced by the number of opportunities the female has to mate, and consequently larger females should

be more likely to mate with multiple males. However, she found no difference in size between females whose litters were sired by one male or by multiple males. Other factors, such as body condition, could be associated with multiple mating. Males in better condition participate in more mating aggregations (Weatherhead et al. 1995; Brown and Weatherhead 1999a). Body condition also might influence female participation in aggregations. Receptivity of female water snakes appears to be under the control of the female. Females may not be receptive continuously throughout the mating season and/or may be receptive only for short periods within the mating season (Weatherhead et al. 1995). Female water snakes observed participating in aggregations on one day may be ignored by males on the next day (pers. obs.). Females in better condition might be receptive more often throughout the mating season and thus more likely to mate with multiple males. Alternatively, females in poor condition may be less able to reject unwanted copulations with males. The number of males encountered by receptive females might also influence the occurrence of multiple mating (Brown and Weatherhead 1999a). Prosser (1999) found no evidence that the incidence of multiple paternity in water snakes increased as the OSR became more male-biased. However, Prosser examined this effect over a small range of OSR, using natural variation in OSR in the wild over three years. I test the prediction that multiple mating should increase with OSR by experimentally varying OSR such that ratios vary from highly female-biased to highly male-biased.

My final objective is to investigate three proposed adaptive consequences of multiple mating. First, multiple mating may insure that a female's ova will be fertilized if a male is sperm limited (Gromko et al. 1984). Thus, singly sired litters may be expected

to have more unfertilised ova. In a previous study of water snakes, there was no difference in the number of unfertilised ova between litters that were singly or multiply sired (Prosser 1999). Second, multiple mating may reduce the proportion of stillborn offspring if mating with several males decreases the risk of having offspring sired by closely related males. Madsen et al. (1992) found that female adders (*Vipera berus*) that mated with more than one male had significantly fewer stillborn young. In water snakes, however, Prosser (1999) found no tendency for singly sired litters to have more stillborn offspring, but her study involved small numbers of unfertilised ova and stillborn offspring. Particularly during one year of my study, female water snakes produced unusually large numbers of unfertilised follicles and stillborn offspring. Given the relatively large number of follicles and stillborn offspring produced by females in this study, I re-examined whether singly sired litters include more unfertilised ova and stillborn offspring. Finally, I examine whether females can increase the diversity of their litters by multiple mating by comparing variation in phenotypic traits of offspring between litters that are singly and multiply sired.

My research builds on several previous studies of reproductive behaviour of the same population of northern water snakes that I studied (Weatherhead et al. 1995; Brown and Weatherhead 1999a; Prosser 1999). In addition to using genetic evidence to determine true patterns of paternity (as did Prosser (1999)), my study differs from these previous studies in one key aspect. I used an experimental rather than a correlational approach. An experimental approach allowed me to manipulate which individuals had an opportunity to mate or compete with each other, whereas correlational studies must rely on natural variation in the factors of interest, and then assume that all the individuals in

the study population have an opportunity to mate or compete with at least a representative sample of the population.

Materials and Methods

Capturing and processing of snakes

Adult male (SVL >42 cm) and female (SVL >55 cm) water snakes were captured in 1997 to 1999 from three beaver marshes located approximately 10 km from the Queen's University Biological Station in eastern Ontario, Canada (45° 37' N, 76° 13' W). Snakes were captured by hand in late April and early May after snakes emerged from hibernation. I attempted to capture individuals prior to observing mating activity in the wild. At the time of capture, I measured snout-vent length (SVL), tail length and mass of each individual. Each individual captured for the first time was marked using a passive integrated transponder (PIT) tag (Anitech Identification Systems, Markham, ON). A blood sample was taken from each individual for use in paternity analysis (see genetic analysis of paternity below).

Mating groups

The general research approach I followed was to establish either small mating groups that were housed in tanks indoors, or larger mating groups that were housed outdoors in enclosures (see details below). My goal was to determine how a number of different factors were associated with mating by water snakes. Given the number of factors of interest, it was not feasible to conduct separate experiments in which I varied each of these factors individually while controlling all other factors. Thus, the approach I

followed was to conduct experiments in which one factor was varied systematically, while simultaneously either minimising variation in other factors, or measuring those other factors so their effect could be controlled statistically after the fact. To determine which factor would be varied systematically in an experiment (i.e., the main effect), I relied on results of previous studies. For example, body condition has rarely been shown to influence mating success of males (but see Shine et al. 2000), whereas male size and tail length have been associated with male success (King 1989; Weatherhead et al. 1995; Shine et al. 2000). Thus, I chose to use two experiments to investigate whether body size or tail length influenced mating success of male water snakes. Additionally, I investigated how size, tail length, and condition influenced success using data from enclosure mating groups in which these variables were not controlled experimentally, but where their singular effects could be investigated statistically.

The first experiment was designed to determine the effect of male size on reproductive success while removing any effect of male condition. I housed each of 12 females in tanks with three males that varied in size but that were similar in condition. Two additional females were housed with two males that differed in size but were similar in condition. At the time of capture, condition was estimated using residuals from a regression of $\ln$ mass on $\ln$ SVL. The maximum difference in SVL between any two males in a mating group was 22 cm (equivalent to 66% of the maximum variation in SVL among all sexually mature males). The maximum difference in condition between any two males in a mating group was equivalent to 23% of the total variation in condition among all sexually mature males.

A second experiment was designed to determine whether tail length influenced male reproductive success, while removing effects of male size and condition. In this experiment, each of 12 females was housed with 3 males that were similar in size and condition. Because male size and tail length are correlated (see results), controlling size of males in experiments also tended to reduce variation in tail length. To increase variation in tail length in this experiment, I included males with shortened tails due to injury in as many mating groups as possible. Tail lengths of males in this experiment varied between 8.3 to 22.9 cm. The maximum difference in condition between any two males in a mating group was 12% of the total variation in condition among all sexually mature males. The maximum difference in SVL was 23% of the total variation in size among all sexually mature males.

To determine the effect of OSR on male reproductive success and multiple mating, I varied the sex ratio of adults in mating groups. Indoors, individual females were housed with one to five males, resulting in the OSR varying between 1:1 and 5:1. Outdoors, four to 12 females were housed with two to 12 males, resulting in the OSR varying between 0.33:1 and 3:1. Snakes used in mating groups outdoors were randomly assigned to mating groups, without consideration of their phenotypes.

To examine whether paternity was biased in favour of the least related males, I used the genetic profiles of males and females in mating groups to establish their relatedness after the experiments were completed. This *a posteriori* approach was necessary because it was not feasible for me to conduct the lab analyses necessary to determine individual genetic profiles in the short time between capturing snakes in the spring and establishing mating groups. To examine whether snakes mated assortatively

and whether characteristics of females influenced their likelihood of mating with multiple males, I simply recorded the phenotypes of males and females used in mating groups and then determined *a posteriori* whether females mated with similar-sized males and whether any characteristics of females were associated with multiple mating.

In mid to late June after females should have ovulated (Bauman and Metter 1977, Feaver 1977, Aldridge 1982), males were removed from tanks and enclosures and released at their sites of capture. Females mated in outdoor enclosures were housed in the enclosures until late July to early August and then were brought into captivity to give birth. Females mated indoors were housed in the same tanks until they gave birth. After giving birth, females were released at their sites of capture. Females that did not give birth by mid-September and that appeared not to be gravid were also released at their sites of capture.

Housing conditions

Six outdoor enclosures measuring ca. 5 m x 6 m served as mating arenas and housing for snakes. Enclosures were constructed of sheet metal (ca. 75 cm high) that was painted to reduce reflection. Each enclosure had a pond (3 m x 3 m x 1 m) which was stocked weekly with minnows from nearby marshes. Vegetation was planted within each enclosure using naturally occurring species found in the study area. Electric fencing around the perimeter of the enclosures discouraged mammals from entering the enclosures. Birds were deterred from entering the enclosures by flagging tape tied to monofilament line strung above the enclosures.

When in captivity indoors, females were maintained in fibreglass tanks (74 cm x 59 cm x 55 cm) lined with artificial grass carpeting or wood shavings. Each tank contained a water dish large enough to allow snakes to submerge. Heating sources (a heating rock or heating coil) were provided in most tanks. In addition, room temperature was maintained above 22 °C, but fluctuated with ambient conditions. Females were fed live or previously frozen fish (e.g., minnows and smelts) *ad libitum* 1-3 times per week.

Genetic analysis and paternity assignment

Blood samples were taken from the caudal vein of snakes using a 0.5 ml insulin syringe fitted with a 28 gauge needle. Approximately 100 µl of blood was taken from adult males and females. Approximately 10 µl was taken from live neonates and a small sample of tissue was taken from stillborn neonates. Samples from adults and neonates were stored in ca. 800 µl and 500 µl of Queen's Lysis Buffer (Seutin et al. 1991), respectively.

I used seven highly variable microsatellite DNA loci to assign paternity to offspring. The primers and characteristics of five of these loci (2, 3, 6, 9b, and 10) are described in Prosser et al. (1999), and the final two loci (110 and 119) are described in Prosser (1999). Details on the isolation and resolution of these loci can also be found in Prosser (1999) and Prosser et al. (1999). Probabilities of exclusion for each locus ranged between 0.21 and 0.82 (Prosser 1999). Prosser (1999) found that four of these loci were typically sufficient to assign paternity to offspring of females mated in the wild where the potential father(s) could have been any of approximately 100 sexually mature males in the population. Because the number of males in mating groups was relatively small (1-

12), I expected that fewer loci would be required to assign paternity to offspring in most litters.

I used a stepwise approach to assign paternity to offspring. I first genotyped each offspring from a litter, their mother, and their potential fathers at a single locus. After determining which offspring could be assigned to a single father based on exclusion, I continued to genotype the remaining offspring, their mother, and the potential fathers at other loci until all offspring could be assigned to a single father. Typically, two to four loci were sufficient to assign paternity to offspring.

There were 120 individuals for which paternity could not be assigned using this method. Ninety-six of these individuals were from seven litters in which none of the males housed with the females sired the litters. This was likely due to females mating prior to me capturing them (see discussion). I did determine the minimum number of males that sired these litters. To do this, I counted the number of paternal alleles at each locus for each litter. I then used the locus with the most paternal alleles to estimate the minimum number of sires for the litter. For example, if there were three paternal alleles, I estimated that at least two males sired the litter. These litters were excluded from analyses requiring data on exact paternity or male reproductive success, but were included in some analyses involving multiple paternity. A potential concern based on this result is whether any offspring whose paternity was assigned to a male from a mating group were actually sired by a wild male that was similar in genotype to the assigned male in the mating group. I consider this possibility unlikely for two reasons. First, most females were captured prior to mating being observed in the wild and thus should not have mated with any other male in the population. Second, using snakes from the same

populations I studied and the same genetic markers, Prosser (1999) estimated that the probability of two males having the same genotype was 0.0015. Thus, there was an extremely small chance that I misidentified the true sires of the litters.

For 12 other individuals, I could not exclude two to four of the males as potential sires. However, for each of these individuals only one of the males had sired all other offspring in their litters, and thus paternity was assigned to these males. Two other individuals could not be assigned because two males could not be excluded and both had sired some offspring in the litter. I arbitrarily assigned these two offspring to the male that sired the greatest proportion of offspring in their litters. Although there is a possibility that I assigned paternity to the wrong male using this method, this error would only affect my estimation of the proportion of offspring sired. Because only two individuals from two different litters were assigned using this method, any error would be small and have little effect on the outcome of my analyses. Ten other individuals from four different litters could not be assigned because their DNA samples would not resolve (i.e., were not visible) on polyacrylamide gels at most or all loci, even after repeated DNA extraction and PCR amplification. Consequently, these individuals could not be genotyped. For two of the four litters, the relative proportion of offspring sired by individual males could not change based on the paternity of these unknown individuals. Thus, I included males from these two litters in analyses requiring the proportion of offspring sired and excluded males from the other two litters from analysis.

Genetic relatedness

To determine whether paternity of offspring varied non-randomly with genetic similarity between the female and the males within mating experiments, I generated relatedness scores (r values; Queller and Goodnight, 1989) using Kinship 1.2 software (Goodnight et al., 1997) for every male and female in each mating experiment pooled across years. In total, calculations were based on 194 individuals genotyped at four loci (2, 3, 6, and 10). Missing genotypes at one of the four loci prevented relatedness values from being generated for two individuals. I originally attempted to genotype all adults at all seven loci, but DNA samples from many individuals did not resolve at one or more of the remaining three loci. To maximize the number of individuals for which relatedness scores could be calculated, I used the four loci for which all but two individuals were genotyped.

Statistical analysis

Non-random success of males

Female water snakes in my study population usually reproduce every second year (Brown and Weatherhead 1997). Despite using only sexually mature females in my experiments, only ca. 46% of laboratory females and 31% of enclosure females gave birth. This problem, coupled with the problem of some of the females that did give birth producing offspring fathered by males other than those in the mating groups, reduced sample sizes. This problem particularly affected the sample sizes for laboratory matings used in determining whether any male attributes affected their mating success. Thus, I used t-tests to examine whether unsuccessful and successful males differed in attributes

for matings conducted in the laboratory. For matings from enclosures where larger samples were available, I used stepwise multiple logistic regression with unsuccessful/successful as the dependent variable and male attributes as the independent variables. A variable entered the model at $\alpha = 0.05$ and stayed in the model at $\alpha = 0.10$. To examine whether OSR influences whether male attributes are important in determining which males are successful, I separated data into mating groups that were conducted at female-biased (0.33-0.40: 1) and male-biased (3:1) OSR and ran this model on these reduced data sets. I used Pearson Product-moment correlations to determine whether the proportion of offspring sired varied with any male attributes.

Because the number of males in mating groups varied and because a female could mate with one or several males in mating groups, resulting in some males being successful and others not, I was concerned that larger mating groups would bias my analysis by contributing more observations than smaller groups. To make the contribution of each mating group equitable, I used mean male attributes of successful and unsuccessful males in my analyses. I used means for both the raw attribute values (e.g., mean absolute SVL) and for sizes of characters relative to the size of those of males in a given mating group (e.g., mean difference of each male's SVL relative to the mating group's average SVL). Results using raw and relative values of male traits were qualitatively similar, so only results from analyses using raw values are presented.

To determine whether successful and unsuccessful males differed in their genetic similarity to females, I used logistic regression with successful/unsuccessful as the dependent variable and mean relatedness to the female as the independent factor. I also calculated relative relatedness values, as above for male characteristics. Again, analyses

using raw and relative values for relatedness produced similar results and I present analyses using only raw values.

To investigate size assortative mating, I used data from enclosure mating groups because both males and females had access to members of the opposite sex. I examined whether there was evidence of a correlation between relative male and relative female size, where relative size was expressed as a rank. For each enclosure, I ranked females and males according to their SVL. However, each enclosure did not contain the same number of snakes, so the maximum rank values (i.e., the largest snake of a each sex) differed among enclosures. This maximum rank value is dependent on the number of snakes of each sex in an enclosure, and not actual SVL. To resolve this, I converted each raw rank score to a rank percentage with the formula: $\frac{(x-1)}{(n-1)} \times 100\%$, where x is the raw rank score and n is the number of snakes being ranked. This method ensured that the lowest rank (smallest snake) always received a rank percentage of 0, the highest rank (largest snake) always received a rank percentage of 100, and the middle rank always received a rank percentage score of 50, regardless of the number of snakes ranked. For females that mated with multiple males, I used the mean of adjusted ranks for the individual males she mated with. I calculated the Pearson Product-moment correlation between adjusted ranks of females and their mates. To determine whether the OSR influenced whether snakes mated assortatively, I calculated separate Pearson Product-moment correlations using data from mating groups in enclosures at female-biased OSR (0.36-0.40:1) and at male-biased OSR (3:1).

Multiple Mating

To determine the incidence of multiple paternity in my study, I included only data from females that were housed with more than one male, and thus had the opportunity to mate with multiple males. To examine whether any attributes of females were associated with their likelihood to mate with multiple males, I used logistic regression with single/multiple paternity as the dependent variable and female size and condition as factors. To determine whether the proportion of multiply sired litters varied with OSR, I calculated the Pearson Product-moment correlation between OSR and the proportion of litters with multiple sires. Finally, I used t-tests to determine whether singly and multiply sired litters differed in the proportion of unfertilised ova or stillborn offspring and in variance in phenotypic traits of offspring.

Analyses were carried out using SAS (SAS 1999) software. For all tests, I assessed whether the data met assumptions of the tests and transformed variables when assumptions were violated. If transformations did not allow data to meet assumptions of parametric tests, I used non-parametric analyses. Significance of all analyses was assessed at $\alpha = 0.05$

Results

Paternity Assignment

Over the three years of the study, 53 litters were produced. The total number of offspring genotyped was 927. Of these, 835 offspring were live and 92 were either stillborn or partly-developed. In addition, 100 unfertilised follicles were produced. I assigned paternity to 821 offspring (88.6%). Of the 106 offspring for which paternity

could not be assigned, 96 offspring were from seven litters that were fathered by males other than those housed with the females. The remaining 10 individuals could not be assigned because their DNA samples failed to resolve at most or all loci (see Methods).

Non-random success of males

I investigated how male SVL, condition, and tail length influenced male reproductive success. Tail length and SVL were correlated ($r = 0.51$, $n = 42$, $p = 0.0006$). To examine the effects of tail length independent of SVL, I used the residuals from a regression of tail length and SVL in my analyses.

Laboratory matings

In the first experiment, I examined the effect of male size on reproductive success while experimentally controlling for male condition. I found that successful males were larger than unsuccessful males ($t = 3.71$, $n = 5$, $p = 0.03$, Fig. 1.1). Successful males had mean SVL ($\pm$ S.E.) of $58.1 (\pm 4.2)$ cm and unsuccessful males had a mean SVL of $48.9 (\pm 1.64)$ cm. In the second experiment, I examined whether tail length of males influenced success while experimentally controlling for male size and condition. Mean relative tail length did not differ between successful and unsuccessful males ($t = -1.95$, $df = 2$, $p = 0.19$). Thus, male tail length does not appear to influence male mating success.

Enclosure matings

I used stepwise multiple logistic regression to determine whether success of males in enclosures was influenced by male size, condition, and relative tail length. Because I

did not control for the phenotypes of males used in mating groups in enclosures, this statistical approach allowed me to examine the effect of a single male attribute on male success while statistically holding other attributes constant. Because stepwise multiple regression is sensitive to correlation among independent factors, I first examined whether there were correlations among these variables. Mean male SVL was not correlated with mean relative tail length ($r = -0.05$, $n = 50$, $p = 0.75$) or mean condition ($r = -0.01$, $n = 50$, $p = 0.96$). However, mean relative tail length was significantly negatively correlated with mean condition ($r = -0.35$, $n = 50$, $p = 0.01$). Because these variables were correlated, I chose to remove tail length from further analyses because results using laboratory matings indicated no effect of tail length. Stepwise multiple logistic regression with mean SVL and mean condition indicated that SVL was the only important factor determining male success (mean SVL: $\beta = 0.12$, $X^2 = 4.34$, $df = 1$, $p = 0.04$. Fig. 1.2; mean condition: $X^2 = 0.67$, $df = 1$, $p = 0.41$). Again, successful males were larger than unsuccessful males.

I had predicted that male characteristics might be more important in determining a male's success when males had to compete against many other males for access to females. At a male-biased OSR (3:1), the mean SVL but not mean condition was important in determining male success (mean SVL: $\beta = 0.43$, $X^2 = 7.92$, $df = 1$, $p = 0.0049$, Fig 1.3; mean condition: $X^2 = 0.47$, $df = 1$, $p = 0.49$). At a female-biased OSR (0.33-0.40: 1), neither mean SVL nor mean condition was associated with the probability of being successful (mean SVL: $X^2 = 0.01$, $df = 1$, $p = 0.90$; mean condition: $X^2 = 0.20$, $df = 1$, $p = 0.66$). Thus, as predicted, male size was more important in determining success when the OSR was male-biased.

Contrary to my prediction that body size and tail length might influence the proportion of offspring sired in multiply sired litters, the proportion of offspring sired did not vary with male size ($r = 0.14$, $n = 38$, $p = 0.39$) or with residual tail length ($r = 0.12$, $n = 38$, $p = 0.47$). Surprisingly, males in poorer condition sired a greater proportion of offspring ($r = -0.32$, $n = 38$, $p = 0.05$). There was no relation between the number of offspring sired and male size ($r = 0.16$, $n = 38$, $p = 0.35$), residual tail length ($r = 0.06$, $n = 38$, $p = 0.71$), or condition ($r = -0.24$, $n = 38$, $p = 0.15$).

My result that the proportion of offspring sired in multiply sired litters was not associated with most male attributes could result if males were sperm depleted. To investigate this possibility I used data on the proportion of offspring sired for individual males in enclosures who sired offspring with more than one female. A male contributing a large amount of sperm to one mating might have little sperm to contribute in a second mating. Similarly, a male contributing a moderate amount of sperm in one mating may have a moderate amount of sperm to contribute to a second. Such a situation would result in an overall negative relation between the proportion sired in one litter and the proportion sired in a second litter. Using reduced major axis regression, there was no association between the proportion of offspring sired (or absolute number) in one litter and that in a second litter (proportion: $r = 0.21$, $n = 9$, $p = 0.58$; absolute number: $r = 0.45$, $n = 9$, $p = 0.22$).

Size assortative mating

I examined whether males and females mated assortatively based on size. Overall, there was no evidence for size assortative mating in enclosures. ($r = 0.07$, $n = 27$, $p =$

0.75). I predicted that snakes might be more likely to mate assortatively when the OSR was male-biased. There was no evidence for size assortative mating when the OSR was male-biased ($r = -0.36$, $n = 10$, $p = 0.31$). However, snakes mated assortatively with respect to size when the OSR was female-biased ($r = 0.67$, $n = 9$, $p = 0.05$, Fig. 1.4).

Effect of genetic similarity between males and females

If females possess some mechanism that allows them to discriminate against genetically similar males, males less genetically similar to females should be more successful. Logistic regression revealed that genetic similarity did not influence male success ($X^2 = 0.13$, $df = 1$, $p = 0.71$). In multiply sired litters, the proportion of offspring sired did not vary with genetic similarity between the parents ($r = 0.04$, $n = 38$, $p = 0.79$). The number of offspring sired also did not vary with the genetic similarity between parents ($r = -0.12$, $n = 38$, $p = 0.46$). Thus, there was no evidence that females mate with males less related to them or bias paternity of their offspring in favour of less related males.

Multiple paternity

In total, I detected multiple paternity in 25 of 46 (54.3%) litters from mating groups in which females had the opportunity to mate with multiple males. The incidence of multiple paternity was 41.2% for litters produced by females mated in the laboratory and 62.1% for litters from females mated in enclosures. All multiply sired litters were sired by 2 or 3 males, resulting in a mean number of sires of 2.4. The proportion of offspring fathered by a single male in multiply sired litters ranged from 0.03 to 0.97. The mean proportion ($\pm$ S.E.) sired by individual males was 0.42 (± 0.05).

I used multiple logistic regression to determine whether the probability of females having their litters sired by multiple males was associated with female size or with female condition. I first determined that female SVL and female condition were not correlated ($r = 0.03$, $n = 46$, $p = 0.84$). A female's probability of having her litter sired by multiple males was associated with her size, but not her condition (female SVL: $\beta = 0.11$, $X^2 = 5.31$, $df = 1$, $p = 0.02$, Fig. 1.5; female condition: $X^2 = 3.04$, $df = 1$, $p = 0.08$). Larger females were more likely to have their litters sired by multiple males. In addition, multiply sired litters were larger than singly sired litters ($t = 2.55$, $df = 51$, $p = 0.01$, Fig. 1.6). On average, multiply sired litters had five more offspring than singly sired litters.

I expected that the number of males encountered by females would influence the occurrence of multiple paternity. However, the proportion of litters that were multiply sired did not vary with the OSR ($r = -0.45$, $n = 12$, $p = 0.14$) or with the absolute number of males in mating groups ($r = 0.03$, $n = 12$, $p = 0.93$).

In my study female water snakes produced unusually high numbers of unfertilised ova and stillborn offspring, particularly in 1999 (Table 1.1). There was no evidence that females mated with multiple males to insure against a male being sperm limited. The mean number of unfertilised follicles did not differ between females whose litters were singly or multiply sired ($t = -0.46$, $df = 51$, $p = 0.65$). There also was no evidence that mating with multiple males decreased the number of stillborn offspring produced by females. In fact, the mean proportion of stillborn offspring was higher in multiply sired than singly sired offspring ($t = 2.43$, $df = 51$, $p = 0.02$, Fig. 1.7). Two females whose litters were multiply sired gave birth to litters in which more than 50% of the offspring were stillborn. Even after removing these litters, there was still a trend for multiply sired

litters to contain more stillborn offspring than singly sired litters ($t = 1.84$, $df = 49$, $p = 0.07$). Finally, I examined whether multiply sired litters were more diverse by comparing variation in phenotypic traits between litters that were singly and multiply sired. Multiply sired litters were more variable in mass and speed than singly sired litters (coefficient of variation (CV) mass: $t = -2.18$, $n = 50$, $p = 0.03$; CV speed: $t = -2.26$, $n = 48$, $p = 0.03$). There were no differences in variation in SVL or condition between singly and multiply sired litters. (CV SVL: $t = -1.67$, $n = 50$, $p = 0.10$, standard deviation condition: $t = -1.48$, $n = 50$, $p = 0.15$). Thus there is some evidence that females increase the variability of their litters by having litters sired by several males.

Discussion

The main objective of this chapter was to examine factors associated with non-random success of male water snakes and multiple mating by female water snakes. This objective required that I accurately assign paternity to offspring. I assigned paternity to 89% of offspring. Although high, this number was lower than I had expected given captive mating groups, primarily because seven females gave birth to offspring sired by males other than those that were housed with them. There are at least two possible explanations for this result. First, females stored sperm across mating seasons. Sperm storage across years is common in several species of snakes (e.g., Fox 1956; Schuett 1982; Gist and Jones 1987) and there is anecdotal evidence that water snakes can store sperm across years (C.S. Berg, pers. comm.). However, the large number of offspring that could be assigned to one or more of the potential fathers suggests that across-year sperm storage is probably uncommon in water snakes. Second, females may have mated prior to

me capturing them from the wild. Although I attempted to catch females prior to the beginning of the mating season, I was not always able to do so because of the short time between when most snakes emerge from hibernation and when they begin to mate (typically 1-3 weeks). I determined that all seven females whose litters were sired by unknown males were captured after the date that mating was first observed in the wild for the year of capture. Thus, these females likely mated prior to me capturing them.

Two of my results demonstrate that water snakes mate non-randomly with respect to body size. First, my results from laboratory and enclosure mating experiments demonstrate that larger males had higher mating success than smaller males. Weatherhead et al. (1995) also found that larger male water snakes had higher mating success. Prosser (1999) found no effect of male size on mating success of male water snakes. However, she found that selection gradients for male size tended to be positive for one marsh population of water snakes, which suggests directional selection for large size in males. Similarly, Brown and Weatherhead (1999b) found no difference in size between males seen in aggregations and those never seen in aggregations, but still found directional selection on body size. I had predicted that when competition among males is high, individual male attributes might be more important in determining mating success. Supporting my prediction, male size influenced mating success when the OSR was male-biased but not when the OSR was female-biased. In Weatherhead et al.'s (1995) study of water snakes, mating success of males in the wild was examined across two years. In that study, large males had higher mating success only in the year when the OSR was more male-biased. Larger male garter snakes also have higher success in mating aggregations (Shine et al. 2000, but see Joy and Crews 1988). Recently, Shine et al. (2000)

investigated the conditions under which larger male garter snakes (*T. sirtalis*) were more successful using controlled mating experiments. They found that the effect of male size on mating success was strongest in experiments where only two males competed for a single female compared to trials in which four and 24 males competed. Their results suggest that the degree of male bias adds another level of complexity in determining how important male attributes are in mating success. In very large aggregations the outcome of mating competition may be less predictable (Shine et al. 2000).

The second result suggesting non-random mating was the evidence of positive assortative mating when the OSR was female-biased. This was contrary to my prediction that snakes would mate assortatively when the OSR was male-biased. My prediction was based on the assumption that large males would monopolize large females, forcing small males to mate with small females. Given that small males have a low probability of siring offspring under male-biased OSR, small males have little opportunity to mate, even with small females. In contrast, when the OSR was female-biased and competition among males was low, large and small snakes had the opportunity to mate. Size-assortative mating under female-biased OSR could then result from larger males out-competing smaller males for access to large females, as I had predicted would occur at male-biased OSR. An alternative explanation is that these patterns are a consequence of females preferring to mate with larger males, rather than males being selective (Prosser 1999). If so, larger females may be able to out-compete smaller females for access to large males, forcing smaller females to mate with smaller males.

An interesting result was that large males did not sire proportionally more offspring in multiply sired litters despite larger males having higher mating success

overall. Thus, the advantage of large size appears to be greater opportunity to mate rather than any advantage post-mating. There was no evidence that the higher mating success of larger males causes them to be sperm depleted, and thus unable to sire a disproportionate number of offspring in multiply sired litters. For males who sired offspring in two litters in the same year, there was no evidence that the proportion of offspring sired in one litter was associated with the proportion sired in a second litter. However, the time between matings may influence a male's ability to replenish sperm. I had no information with which to address this in my analysis but this certainly provides a potential avenue for future study on fertilization success of males.

Reproductive success of males was not influenced by their relatedness to the females with which they were housed, although it is unlikely that females could assess genetic similarity of males prior to mating (Zeh and Zeh 1997). However, I also found no evidence that paternity of offspring of females that produced litters that were multiply sired was biased in favour of the least genetically related male(s). Although dispersal of water snakes between marsh populations is low in my study area, these populations are not inbred (Prosser et al. 1999). Thus, selection favouring female water snakes that discriminate against males who are genotypically similar to themselves may not be intense. Although the available evidence for water snakes suggests that females do not discriminate against related males, a better method to assess this would be to vary the degree of relatedness experimentally within mating groups using males and females for which relatedness is known. I did not know the relatedness of females and males when I established mating groups so variation within groups was not maximised.

Over half (54.3%) of all females housed in captivity with multiple males produced litters that were sired by more than one male. It is possible that housing females in small mating arenas increased the incidence of multiple paternity in this study because males did not have to spend time searching for females. However, a recent study of this population of water snakes showed that 54.1% of litters from females mated in the wild were multiply sired which is similar to my result (Prosser 1999). Thus, there is no evidence that captive conditions influenced the incidence of multiple paternity in this study.

The only factor associated with multiple paternity was female size. Larger females were more likely to have their offspring sired by multiple males. Presumably because larger females produce larger litters, I also found that litter sizes were higher in multiply sired litters than in singly sired litters. Prosser (1999) found no effect of female size on the incidence of multiple mating by female water snakes. However, a reanalysis of her data indicated that the number of sires per litter increased with maternal size (Prosser, Weatherhead, Gibbs and Brown, unpubl. data). These results are consistent with the prediction that larger females would be more likely to have litters sired by multiple males as a result of them being more attractive due to their high fecundity (Prosser 1999). An alternative explanation is that multiple paternity is more likely in larger litters simply because more ovules are available to be fertilised. Consequently, when a large female mates with males who contribute different amounts of sperm, the male(s) who contributes less sperm has a higher probability of fertilising some ovules.

One proposed advantage to multiple mating is that it may insure that all a female's ova are fertilised if one copulation is insufficient to fertilise all ova (Gromko et

al. 1984). Inconsistent with this hypothesis, I found that the number of unfertilised ova did not differ between singly and multiply sired litters. Another proposed benefit of multiple mating is that multiple mating decreases the possibility that a genetically related male will sire all of a female's offspring. Inconsistent with this hypothesis, I found that multiply sired litters had proportionally more stillborn offspring than singly sired litters. One potential cost of multiple mating is that it exposes females to an increased risk of contracting a disease (e.g., Lombardo 1998, 2000; Westneat and Rambo 2000) that could have resulted in abnormal development of offspring. The fact that stillborn offspring were more prevalent in one year of my study lends some support to the idea that this resulted from disease. Another proposed benefit of multiple mating is that females may increase the genetic diversity of their litters by having their offspring sired by several males. Consistent with this hypothesis, I found that the variance of some phenotypic traits was higher in multiply sired litters than singly sired litters. If producing variable offspring increases mean survival of a female's offspring, females may benefit from mating with several males.

The next step is to determine whether females increase their fitness by mating with large males or multiple males by comparing fitness of offspring produced by females exhibiting these behaviours. In other taxa, such as birds, there are numerous examples of offspring fitness being affected by female mating decisions. For example, Petrie (1994) showed that peahens (*Pavo cristatus*) that mated with peacocks with more elaborate trains gave birth to offspring with higher growth and survival rates. Similarly, female great tits (*Parus major*) that mated with males with larger breast stripes gave birth to male offspring that were more viable (Norris 1993). In mammals, survival to yearling

age for juvenile ground squirrels (*Spermophilus richardsonii*) tended to increase with the number of mates of their mothers.

Relatively few studies of reptiles have examined the fitness consequences of female mating behaviour. This is presumably due to the difficulty in studying hatchlings and neonates due to their being small, elusive, and cryptically coloured. Despite evidence indicating that reproductive success varies with male phenotypes, there has been no emphasis on determining how offspring fitness might be affected. This is probably because the mechanism implicated in producing differential male success is male competition rather than female choice (Olsson and Madsen 1995). However, I suggest that the consequences for offspring of females passively mating with males that win competitions or actively choosing males could be similar. Females who actively choose to mate with males based on a phenotypic trait presumably do so because this trait reflects male quality. Similarly, males who possess a trait that makes them competitively superior in mating competitions presumably are of higher quality than males that lose competitions.

Two studies of reptiles suggest that females receive substantial indirect fitness benefits from multiple mating. In sand lizards (*Lacerta agilis*), Olsson et al. (1994b) found that the number of males with which a female mated was significantly positively correlated with the hatching success of her eggs and with the proportion of her offspring that survived one year. In adders, offspring born to females that mated multiply had higher survival to one year (Madsen et al. 1992). In Chapter Four I examine the fitness consequences of non-random success of males and multiple mating by female water snakes, by monitoring over-winter survival of their offspring.

Table 1.1 The number of unfertilised ova and stillborn/partly developed offspring in northern water snake litters from 1997 to 1999.

	Year		
	1997	1998	1999
Unfertilised ova	27	30	43
Stillborn/Partly developed offspring	6	14	72

Figure 1.1. The mean ($\pm$ S.E.) SVL of male northern water snakes that were successful and unsuccessful in siring offspring.

Figure 1.2. Relationship between size of male water snakes and their probability of siring offspring. Male size is represented by mean sizes of males from a mating group that were successful or not successful in siring offspring.

Figure 1.3. Relationship between size of male water snakes and their probability of siring offspring. Data are from mating groups with male-biased OSR. Male size is represented by mean sizes of males from a mating group that were successful or not successful in siring offspring.

Figure 1.4. Relationship between the rank of SVL of female water snakes and the rank of SVL for their mates at female-biased operational sex ratios.

Figure 1.5. Relationship between size of a female and the probability of her litter being sired by multiple males.

Figure 1.6. Mean ($\pm$ S.E.) litter size of singly and multiply sired litters.

Figure 1.7. The mean ($\pm$ S.E.) proportion of stillborn or partly-developed offspring in singly and multiply sired litters.

CHAPTER TWO
MATERNAL INFLUENCES ON SIZE AND SEX OF OFFSPRING IN
NORTHERN WATER SNAKES, *NERODIA SIPEDON*

Introduction

The phenotype of an individual at birth has important implications for its future growth, reproduction, and survival (Roff 1992; Stearns 1992). Offspring phenotypes are products of genes and genotype-environment interactions (Roff 1992). However, there is also considerable evidence that offspring phenotypes may be subject to maternal effects, defined as any effect acting through the mother on the phenotype of her offspring (General Introduction). Because a female's fitness is determined by the number of her offspring that survive to reproduce, females that influence the phenotypes of their offspring in ways that enhance their probability of surviving and reproducing will also increase their own fitness. Thus, maternal effects should generally be adaptive (Mousseau and Fox 1998).

Evidence for maternal effects comes from studies that document correlations between maternal attributes and offspring phenotypes where none are expected (Meffe 1990; Bernardo 1996a,b; Weatherhead et al. 1999). For example, life history models that examine how females should invest in size versus number of offspring predict that, for a given population, there is an optimal investment per offspring that maximizes a female's fitness (Smith and Fretwell 1974). Consequently, offspring size is expected not to vary within populations, and thus, not to vary with any characteristics of females within those populations. Contrary to this expectation, however, correlations between offspring size and maternal attributes are common (Bernardo 1996a,b). Similarly, offspring sex ratios

have been shown to vary with the attributes or circumstances of their mothers (e.g., size, social status) (Trivers and Willard 1973; Godfray and Werren 1996), contrary to the expectation that sex ratios should be equal in sexually-reproducing organisms with genotypic sex determination (Speith 1974; Charnov 1982). These examples indicate that a mother's contribution to her offspring is not limited only to her genetic contribution. My goal in this chapter is to investigate maternal effects on offspring size and sex ratio variation in northern water snakes, *Nerodia sipedon*.

The northern water snake is a good subject to investigate maternal influences on offspring phenotypes for several reasons. Parental care is absent in snakes, so maternal influences are limited to those occurring prior to parturition. In addition, details of the reproductive cycle and embryogenesis are well studied, providing insight into where mothers have most influence on offspring size. Water snakes emerge from hibernation in late April and mate during May and early June (Weatherhead et al. 1995). Follicles develop throughout May and June and are ovulated in mid- to late-June (Bauman and Metter 1977; Feaver 1977; Aldridge 1982). Investment of energy into follicles is complete at ovulation, but females are able to provision embryos with water and minerals across the placenta (Conway and Fleming 1960; Stewart and Castillo 1984; Stewart 1989). Typically, gestation continues until late August or early September and females give birth to an average of 19.5 offspring (Brown and Weatherhead 1997).

Maternal influences on offspring size variation

In northern water snakes, maternal influences on variation in offspring size have recently been investigated by Weatherhead et al. (1999) for the same population of water

snakes that I studied. Weatherhead et al. (1999) examined how offspring size varied among females in relation to maternal phenotypes and intake of energy. They found that larger females and females in better condition produced larger litters of smaller offspring. At a given litter size, larger females and females in better condition produced larger offspring. They also found that females used energy gained while follicles are developing (May-mid June) to increase the size of their offspring and their litters. Finally, Weatherhead et al. (1999) found that larger litters were born earlier. Because litter size was also related to female size and condition, larger females and females in better condition gave birth to larger litters of smaller offspring that were born earlier in the summer. Weatherhead et al. (1999) proposed that being born earlier might allow smaller neonates to realize the same fitness as larger neonates born later, if earlier birth allows neonates more time to forage and store energy prior to hibernation. Consequently, these results may provide evidence that female water snakes adaptively manipulate the tradeoff between offspring size and litter size as a function of their body size and condition (Weatherhead et al. 1999). In several other species of snakes larger litters are born earlier (*S. dekayi*: King 1993; *T. sauritus* and *T. sirtalis*: Sommerer 2000).

My first objective in this chapter is to verify maternal effects on offspring size shown by Weatherhead et al. (1999) by examining whether offspring size varies with maternal size, maternal condition, litter size, timing of birth, and food availability. My goal in replicating these analyses was twofold. First, for their own sake, replicating studies is important since patterns of variation may differ among populations or, in this case, among years. Second, patterns of offspring size variation in my data have consequences for interpreting results in other chapters. For example, in Chapter Four, I

examine the fitness consequences for females of varying their investment into offspring. Consequently, it is important to verify that offspring size varies significantly among females used in this study.

I use two methods to investigate the effect of food availability on variation in offspring size and litter size. First, I compare variation between females maintained in captivity and females in the wild, taking advantage of the fact that females maintained in captivity should have had greater access to food. Second, I compare variation between females that were maintained on a high-energy diet with those on a low-energy diet. Based on the results of Weatherhead et al. (1999) indicating that female water snakes invest energy gained prior to ovulation into their litters, I expected that offspring size and/or litter size would be higher for captive females and females on the high-energy diet.

Weatherhead et al. (1999) considered only how mean offspring size differed among litters. However, it is possible that variance in offspring size also differs when comparisons are made across particular groups of females (e.g., females exposed to different levels of food availability). Only one study of snakes has compared the variance in reproductive characters among females' litters. Seigel and Ford (1992) found no difference in variance of offspring size or litter size between female garter snakes (*T. marcianus*) maintained on a high- or low-energy diet. My second objective in this chapter is to investigate whether food availability influences the variance of offspring size and litter size by comparing variance in offspring size and litter size between captive females and females from the wild, and between females on high- and low-energy diets. The expectation from optimal offspring size theory is that there should be little variance in

offspring size among females (Smith and Fretwell 1974; Meffe 1990). However, Weatherhead et al.'s (1999) result showing that mean offspring size varies with energy intake by female water snakes prior to ovulation provides a basis for expecting that the variance may also be affected. I assumed that because captive females and females on the high-energy diet have greater access to food, there was more potential for them to differ from one another in their food intake. Thus, these females were also more likely to differ from each other in how much they invested in individual offspring compared to wild females and females on the low-energy diet. Therefore, I predicted that variance in offspring size and litter size for captive females and females on the high-energy diet would be higher than for wild females and females on the low-energy diet. I also examine variation across five years for females from the wild in order to investigate general patterns of offspring size variation in *N. sipedon*.

Offspring size often varies considerably within the litters of individual females, as well as among their litters (e.g., Crump 1981; Brodie and Ducey 1989). Within-litter variation in offspring size may be a simple consequence of females being unable to control precisely how energy is allocated to their offspring (Meffe 1990). Alternatively, mothers may influence how much the size of their offspring varies within their litters. Females might increase offspring size variation when the environment is unpredictable, increasing the likelihood that at least some of those offspring (the largest individuals) survive when conditions are bad (Capinera 1979; Kaplan and Cooper 1984, 1988; Seigel and Ford 1992; but see McGinley et al. 1987). Crump (1981) showed that female tree frogs breeding in temporary ponds produced clutches of variably-sized eggs, whereas females breeding in permanent ponds produced clutches of similarly-sized eggs. In

snakes, within-litter variation in offspring size has been reported for several species (Brodie and Ducey 1989; Seigel and Ford 1992; Sommerer 2000), including northern water snakes (Weatherhead et al. 1999). In the genus *Thamnophis* alone, there is evidence both for and against maternal phenotypic influences on within-litter variation. Sommerer (2000) found that larger mothers produced litters of more variable offspring in *T. sauritus*, but not in *T. sirtalis*, and Seigel and Ford (1992) found no evidence that variation increased with female size in *T. marcianus*.

The main reason that it is unclear whether maternal attributes regularly contribute to variation in offspring size within snake litters is that little research has been done on this topic. Thus, my third objective is to examine whether maternal attributes and the maternal environment contribute to within-litter variation in offspring size. I predict that within-litter variation will increase with litter size because females might have less control over the size of follicles as litter size increases (Seigel and Ford 1992). I also predict that within-litter variation in offspring size will increase with mass gain of females prior to ovulation, because females might have less control over how energy is allocated to offspring when they have more energy to invest. Finally, I predict that offspring born late in the season will be more variable. Because environmental uncertainty seems likely to increase as the season progresses, females that give birth later might increase variability of their offspring to increase the possibility that some of those offspring will survive if conditions are poor (e.g., early onset of cold weather).

Variation in offspring size among females must be a consequence of both genetic and ecological factors. Some insight into the relative importance of these factors can be gained by determining whether individual females consistently produce similar-sized

offspring across their litters. Low consistency across an individual female's litters would suggest that ecological factors have the strongest influence on offspring size, or that offspring size varies as a function of female age (Luiselli et al. 1996; Madsen and Shine 1996). High consistency in offspring size across a female's litters could indicate either that individual females are genetically predisposed to produce offspring of a given size, or that ecological differences (e.g., foraging efficiency) among females are consistent. The relative importance of these two factors might be indicated by the extent to which individual consistency varies over time. Litters produced closer in time should be more likely to have been affected by similar ecological conditions than would litters that are more temporally remote. Thus, my fourth objective is to determine how consistent females are in the size of the offspring they produce across their litters, and whether that consistency declines as the time between litters increases.

Maternal influences on offspring sex ratios

In sexually reproducing organisms, females are expected to produce an equal number of sons and daughters, on average, because of the random segregation of sex chromosomes during meiosis (Speith 1974). Although in many organisms sex ratios are equal, sex ratios that differ consistently from equality have been documented in a number of taxa (reviewed by Charnov 1982) and biases may be due to females adaptively manipulating sex ratios of their offspring (Godfray and Werren 1996).

In snakes, sex is determined chromosomally and females are the heterogametic sex, so there is some potential for females to vary sex ratios of their offspring. Weatherhead et al. (1998) recently tested predictions about how northern water snakes

might adaptively vary sex ratios of their offspring. Their predictions were based on the assumption that, because water snakes exhibit female-biased sexual size dimorphism (Weatherhead et al. 1995), larger size should be more beneficial to females. Consequently, they predicted that any trait that allows offspring a head start on growth should result in litters being disproportionately female. Supporting this prediction, females that produced larger offspring produced more daughters (Weatherhead et al. 1998). Weatherhead et al. (1998) did not find any association between sex ratio and timing of birth, despite their prediction that earlier born offspring should be disproportionately female. Weatherhead et al. (1998) also found that larger mothers produced disproportionately more daughters, contrary to their prediction that there would be no association between sex ratio and maternal size, because maternal size does not influence offspring size. However, a later study of this population of water snakes showed that larger females do produce larger offspring when litter size is held constant (Weatherhead et al. 1999), so the earlier finding that larger females produced more daughters (Weatherhead et al. 1998) was consistent with females adaptively varying the sex of their offspring. My fifth objective in this chapter is to investigate whether sex ratios in *N. sipedon* vary predictably with attributes of the females, their offspring, or their litters, as was shown by Weatherhead et al. (1998). I reconsider whether larger females produce more daughters, whether females that produce larger offspring produce more daughters, and whether females that give birth earlier produce more daughters.

The production of litters that are biased toward one sex, regardless of the basis for that bias, could have implications for the phenotypes of the offspring. In placental animals, developing male and female embryos are exposed to circulating sex hormones

of both sexes as a result of the association between fetal membranes and the placenta (Osypka and Arnold 2000). There is some evidence that the sex ratio of offspring may influence the expression of sexually dimorphic phenotypes and behaviours in both sexes, apparently as a consequence of the effect of sex hormones on development (Flores et al. 1994). In male-biased litters females develop in an environment with high levels of circulating androgens, whereas in female-biased litters males develop in an environment of high levels of circulating estrogens. Respectively, these conditions could result in females becoming masculinized and males becoming feminized (Osypka and Arnold 2000).

Recently, Osypka and Arnold (2000) examined the effect of litter sex ratio on tail length in two populations of garter snakes, *T. elegans*. Male garter snakes have longer tails than females, and tail length in neonates can be increased by testosterone administered during development. Osypka and Arnold (2000) predicted that males would have shorter tails (become feminized) in female-biased litters and females would have longer tails (become masculinized) in male-biased litters. Contrary to their expectations, the only relationship Osypka and Arnold (2000) found between litter sex ratio and tail length was that in one of the two populations, females from male-biased litters were more feminized. Although this result was contrary to prediction, and occurred in only one of the two populations, the fact that Osypka and Arnold (2000) found a significant relationship suggests that it is worthwhile examining this effect in different populations and in different species.

Similar to garter snakes, water snakes are placental and exhibit male-biased dimorphism in tail length (Conway and Fleming 1960; Weatherhead et al. 1998). Thus,

they are good candidates to examine how the expression of this trait is influenced by litter sex ratios. My final objective in this chapter is to investigate whether tail length of females increases (i.e., becomes masculinized) with increasing proportions of males in litters and whether tail length of males decreases (i.e., becomes feminized) with increasing proportions of females in litters.

Materials and Methods

I conducted this study from April to October of 1997 to 1999 at the Queen's University Biological Station (QUBS) in eastern Ontario, Canada (45° 37' N, 76° 13' W). I also used data from 1994 to 1996 that were collected by other researchers at the same study site. Adult females (>55 cm SVL) were captured from three beaver marshes and maintained in captivity until parturition. These females were captured at different times during the active season relative to when their follicles were formed and ovulated and were assigned to different housing conditions. This method resulted in three groups of females. 'Free-living' females were captured in late July and early August and were kept in captivity until they gave birth. These females were in the wild during May and June when their follicles were formed and ovulated. 'Captive' females and 'enclosure' females were captured in late April or early May, shortly after they emerged from hibernation. These females were kept indoors in tanks or in outdoor enclosures, respectively, throughout the entire active season, and thus were in captivity (indoors or outdoors) when follicles were formed and ovulated. Both captive and enclosure females were mated in captivity (in tanks or enclosures) by housing them with several males. Because captive and enclosure females were captured over a short duration, their time in captivity was

similar among females. Thus, any effect of captivity on offspring size should have been similar among captive and enclosure females. Enclosure females were moved indoors in late July and early August and kept there until they gave birth. Free-living and captive females were studied in all years of the study, whereas enclosure females were studied only from 1997-1999.

Data on captive and free-living females and the size of their offspring and litters that were collected during 1994-1997 appear in Weatherhead et al. (1999) and were available for use in this study. In four instances, data on females and their litters appearing in Weatherhead et al. (1999) were from successive litters from two individual females. I removed these litters from Weatherhead et al.'s (1999) dataset prior to my analysis to avoid problems of non-independence of data (Sokal and Rohlf 1981). Data on litter sex ratios collected from 1994-1997 appear in Weatherhead et al. (1998). Weatherhead et al. (1998) also included data on litter sex ratios from 14 free-living females collected in 1990 at the same study site. I also included those data in my analyses involving sex ratios.

At the time of capture, total length, tail length, snout-vent length (SVL), and mass of each female were measured. Females in the free-living sample from 1994 to 1996 were measured several times during the active season. In addition, captive and enclosure females were also measured several times over the active season. For these females, I calculated change in mass during the period when follicles were developing (May - mid June). During 1997-1999, free-living females were not captured until just prior to parturition, precluding measurements of change in mass. Thus, these free-living females were excluded from analyses using measures of mass increase. The time between

measurements varied among females because I could not capture all females on the same day. To standardize measurements of mass increase among females, I divided mass increase by the number of days between captures and expressed the increase as 'growth rate'. I estimated condition of females in May as their residual from a regression of $\ln$ mass on $\ln$ SVL. Because I was unable to calculate condition in May for free-living females in 1997-1999, I excluded these females from analyses involving condition.

'Enclosure' females were maintained in six outdoor enclosures measuring ca. 5 m x 6 m. Enclosures were constructed of sheet metal (ca. 75 cm high) that was painted to reduce reflection. Within each enclosure a pond was dug and was stocked weekly with minnows from nearby marshes. Ponds were stocked with similar numbers of minnows. The ponds measured ca. 3.0 x 3.0 m and were ca. 1.0 m deep. Vegetation was planted within each enclosure using naturally occurring species found in the study area. Electric fencing was placed around the perimeter of the enclosures to discourage mammals from entering the enclosures. Birds were deterred from entering the enclosures by using flagging tape strung above the enclosures.

When in captivity indoors, females were maintained in fibreglass tanks (74 cm x 59 cm x 55 cm) lined with artificial grass carpeting or wood shavings. Each tank contained a water dish large enough to allow the snake to submerge. Heating sources (a heating rock or heating coil) were provided in most tanks. In addition, room temperature was maintained above 22 °C, but fluctuated with ambient conditions. Females were fed live or previously frozen fish (e.g., minnows and smelts) *ad libitum* 1-3 times per week.

I manipulated the amount of food available to females in enclosures, beyond what the females could capture in the ponds. Females were assigned to either a low-energy or

high-energy diet. Females on the high-energy diet were hand fed fresh or previously frozen fish twice per week. I attempted to feed each female at least six fish (weighing ca. 6 - 15 g each) at each feeding. Females on the low energy diet did not receive supplemental food in 1997. In 1998 and 1999, females on the low energy diet were fed twice per week but were only fed ca. three minnows at each feeding.

During August and September, tanks were checked several times per day for signs of parturition. Females were weighed throughout August and were weighed and measured within 12 hours following parturition. Litter mass was calculated as the difference between prepartum and postpartum mass. Neonates were weighed and measured within 48 hours after birth. Neonates (including stillborns) were sexed by probing for the presence of hemipenes. The accuracy of this method was assessed for a subsample of individuals by everting the hemipenes of males and attempting to do so for females. In addition, tail lengths of individuals confirmed to be male were approximately 5 mm longer than those for females. Undeveloped follicles and stillborn neonates were counted and weighed and included in my determination of litter size and litter mass. Partly-developed offspring and follicles were not included in measures of offspring size, but fully developed stillborn offspring were included in these measures. I scored the date of birth as the number of days after 1 January (i.e., Julian days). Birth date is not a precise index of the length of gestation unless the date of ovulation is known. However, because captive females gave birth earlier than enclosure or free-living females (see below) due to their exposure to warmer and less variable temperatures during gestation, birth date appears to be a reasonable estimate of the length of gestation. The research reported here was approved by the Carleton University Animal Care Committee.

Statistical Analysis

In several instances, I wished to verify maternal effects on offspring/litter size and sex ratios that had previously been reported in Weatherhead et al. (1999) and Weatherhead et al. (1998), respectively. My goal was to determine whether results using my data were consistent with the results of these previous studies. When the analysis of my data alone revealed relations similar to that of Weatherhead et al. (1998, 1999), I pooled my data with theirs and provided results only for the combined datasets. To judge whether relations were similar, I examined (1) whether the magnitude and direction of each relation was qualitatively similar and (2) whether the significance test on each relation yielded consistent conclusions. However, because my dataset was smaller and had a lower probability of detecting significant relations due to lower power, I also pooled data when my results were not significant, but the magnitude and direction of the relation appeared qualitatively similar. If my data produced results that were dissimilar in magnitude and direction to that found by Weatherhead et al. (1998, 1999), I present results for my data independently. In these cases, I examined my data further to determine why my results differed from those of the previous study.

Analyses involving offspring size were based on mean values for each litter. To investigate the factors contributing to within-litter variation in offspring size, variance within litters was calculated using the method of Sokal and Braumann (1980) for comparing coefficients of variation (CV) among sample or treatment groups. This method involved calculating the deviations of each measurement from the mean of the variable as $|\ln Y_{ij} - \overline{\ln Y_i}|$, where $\ln Y_{ij}$ is the logarithm of offspring size of the j^{th} individual and $\overline{\ln Y_i}$ is the mean of the logarithm for that litter. I then calculated the mean

of these deviations so that variation in offspring size within each female's litter was represented by a single CV.

I also used this method to investigate among-litter variation across treatment groups differing in food availability. I compared among-litter CV for females across rearing conditions (captive, enclosure, and free-living females), across diets (high energy and low energy), and across years (1994-1998). To calculate CV among litters, I used the formula above but used the deviations of each female's mean offspring size and litter size from the mean offspring size and litter size for the particular rearing condition, diet, or year. Again, this method provided a single CV for each female's litter.

In analyses involving within- and among-litter variation in offspring size with respect to food availability, I used treatment group as the predictor variable, the variable of interest describing offspring size (e.g., mean, among-litter CV, within-litter CV) as the response variable, and maternal size and litter size as covariates. For analyses involving comparisons among years for free-living females, 1999 data were removed because the small sample size ($n = 2$) precluded calculations of parameter estimates.

I used two methods to determine whether individual females were consistent in their reproductive output across litters. First, I used ANCOVA with maternal identification number as the predictor variable, the variable of interest denoting reproductive output (e.g., offspring size, litter size) as the response variable, and maternal size as a covariate. Second, I regressed females' first litters against their last litters for the variable of interest using reduced major axis regression. Reduced major axis regression treats both variables as predictor variables and assumes there is error in each variable (Sokal and Rohlf 1981). To control for changes in maternal size between litters, I used

the residuals from a simple linear regression of maternal size against the variable of interest as my independent and dependent variables in the reduced major axis regression. If females are consistent in their reproductive output, the slope of a regression on these residuals will be significantly positively related. Confidence intervals (95%) were used to determine whether slopes differed significantly from zero.

Each of these methods has an advantage that the other does not. The first method allows all of a female's litters to be used in the analysis, whereas the second allows only two litters to be incorporated. The second method allows for a graphical representation of the relationship, whereas the first does not. I also used similar methods to investigate whether time between an individual's litters influences consistency in reproductive output. In the first method, I divided females into two groups based on whether their litters were separated by 1 or 2 years or by 3 or 4 years. Only a female's first and last litters were used to prevent individuals from falling into both categories. Separate ANCOVA's (see above) were run for each group of females. In the second method, I calculated the absolute value of the difference between the pairs of residuals from the first and last litters used above. This difference represents how similar each female is in her reproductive output across her litters, with smaller differences indicating greater consistency. I then regressed these differences against the number of years between the two litters. This allowed me to determine whether the consistency of the trait of interest varied with the time between litters. Significance of these regressions was assessed by determining whether the slopes of the lines differed from zero.

I calculated sex ratio as the proportion of males within a litter. Sex ratios were arcsine-transformed to normalize the distributions (Sokal and Rohlf 1981). In analyses

examining how sex ratio varies with offspring size, offspring SVL was used as a measure of size because my tests were based on the assumption that increased length at birth should benefit females more than males. To determine how sex ratio influenced tail length of male and female offspring, I used tail length relative to SVL ('relative tail length') to control for differences in size among offspring.

Analyses were carried out using SAS (SAS 1996) and Statistica (Statsoft 1993) software. I used parametric statistics and transformed the response variable when data did not meet the assumptions of parametric tests. Nonparametric statistics were used when transformations failed to result in data that met assumptions of parametric tests. Significance was assessed at $\alpha = 0.05$.

Results

Data were collected on 52 free-living females and their litters (11 in 1994, 8 in 1995, 18 in 1996, 8 in 1997, 5 in 1998, 2 in 1999), 33 captive females and their litters (3 in 1994, 9 in 1995, 5 in 1996, 7 in 1997, 4 in 1998, 5 in 1999) and 18 enclosure females and their litters (7 in 1997, 4 in 1998, 7 in 1999). Measurements of females, offspring, and litters are provided in Table 2.1. In the spring, reproductive females ranged in mass from 163 to 506 g and in SVL from 63.3 to 89.8 cm. Litter size ranged from 7 to 40. Overall, the range in size of neonates (excluding stillborn offspring) was 1.5 to 6.4 g for mass and 11.3 to 20.1 cm for SVL. Among litters, mean offspring mass ranged from 3.0 to 5.8 g and mean SVL ranged from 14.0 to 18.9 cm. Within litters, the largest range in offspring size (excluding stillborn offspring) was 2.7 to 6.3 g in mass and 12.6 to 19.6 cm in SVL. Neonate mass and SVL were highly correlated ($r = 0.84$, $n = 103$, $p < 0.0001$).

Because mass is a more direct measure of maternal investment in offspring, I used offspring mass as my measure of offspring size in most analyses and I only differentiate between mass and SVL when both were used in analyses.

Captive, enclosure, and free-living females differed significantly in SVL ($F_{2,87} = 7.09$, $p = 0.001$). Enclosure females were longer than free-living females, but captive and enclosure females did not differ in length. In addition, captive and enclosure females were significantly heavier than free-living females, but captive and enclosure females did not differ in mass ($F_{2,86} = 5.22$, $p = 0.007$). These differences likely reflect a sampling bias as a result of larger females preferentially being chosen for captive and enclosure samples in order to maximize the number of females that gave birth.

Captive females gave birth earlier than enclosure and free-living females ($F_{2,100} = 35.42$, $p < 0.0001$), as expected because captive females should have experienced warmer and less variable thermal conditions during gestation. Mean birth date did not differ between enclosure and free-living females.

Maternal Effects on Variation in Offspring Size and Litter Size

Among Litter Variation

I examined the effect of maternal phenotype and environment on offspring size and litter size. Several of the maternal effects found by Weatherhead et al. (1999) were also apparent in my analysis. Larger females produced larger litters ($r = 0.57$, $n = 103$, $p = 0.0001$, Fig. 2.1), but not larger offspring ($r = 0.06$, $n = 103$, $p = 0.52$). When the effect of litter size was removed using partial correlation, maternal size was positively related to offspring size ($r = 0.32$, $n = 103$, $p = 0.0009$). Thus, at a given litter size, larger females

produced larger offspring. I also found evidence of a tradeoff between litter size and offspring size. Larger litters were composed of smaller offspring ($r = -0.35$, $n = 103$, $p = 0.0008$, Fig. 2.2). When the effect of maternal size was removed using partial correlation, this relationship became stronger ($r = -0.44$, $n = 103$, $p = 0.0001$). Offspring size did not vary with maternal condition ($r = 0.02$, $n = 89$, $p = 0.84$), but females in better condition produced larger litters ($r_s = 0.34$, $n = 89$, $p = 0.001$, Fig. 2.3).

In contrast to Weatherhead et al. (1999) who found no relation between offspring size and birth date, I found that offspring size decreased with birth date ($r = -0.43$, $n = 34$, $p = 0.01$). I investigated why my data may have differed from those of Weatherhead et al. (1999). One difference between this study and that of Weatherhead et al. (1999) was that my analysis included data from enclosure females, whereas Weatherhead et al.'s (1999) study used data only from captive and free-living females. If the relationship between offspring size and litter size for enclosure females was different from that for captive and free-living females, this may have accounted for my results differing from those of Weatherhead et al. (1999). After removing data from enclosure females, offspring size was no longer related to birth date ($r = -0.40$, $n = 16$, $p = 0.12$). Thus, the inclusion of enclosure females appears to account for the difference in results between the two studies.

In contrast to Weatherhead et al. (1999) who found a negative relation between litter size and birth date, I found no relationship between litter size and birth date ($r = 0.27$, $n = 34$, $p = 0.12$). After removing data from enclosure females (as above), there remained no relation between litter size and birth date ($r = 0.04$, $n = 16$, $p = 0.88$). Thus, the inclusion of enclosure females does not account for the difference in results between

studies. Weatherhead et al. (1999) noted that their result was due in part to captive females tending to give birth earlier and to larger litters than free-living females. An examination of both datasets revealed that litter sizes for captive females in 1998 and 1999 appeared to be smaller than for captives used by Weatherhead et al. (1999). After controlling for maternal size, litter sizes of captive females differed among years ($F_{5,26} = 4.62$, $p = 0.004$). Litter sizes in 1998 and 1999 were significantly smaller than in 1995, 1996, and 1997, but did not differ from litters in 1994. Thus, the difference in results between the two studies may be a consequence of litter sizes being smaller for captive females in this study.

Despite my not finding a correlation between timing of birth and litter size, I found that females in better condition (i.e., females that produce larger litters) gave birth earlier ($r = -0.27$, $n = 89$, $p = 0.01$, Fig. 2.4). Although larger females also gave birth to larger litters, I did not find a relationship between maternal size and timing of birth ($r = 0.03$, $n = 103$, $p = 0.78$).

I predicted that because females maintained in captivity should have eaten more than enclosure or free-living females, captive females should have gained more energy prior to ovulation and invested this energy in their litters. I also predicted that this would result in higher variance in offspring size among litters for captive females. Holding females in captivity and providing them with relatively unlimited food did result in captive females gaining more mass prior to ovulation than enclosure and free-living females ($F_{2,54} = 4.72$, $p = 0.01$). Contrary to my expectations, however, captive females did not produce larger offspring or larger litters compared to free-living females or enclosure females (Table 2.2). There was a tendency for offspring mass to differ among

groups ($p = 0.07$, Table 2.2), but mass was lowest for enclosure females and highest for free-living females. Enclosure females did produce offspring that were significantly shorter in SVL than offspring of captive and free-living females, but offspring SVL was similar for captive and free-living females. There were no differences in the variances of offspring mass, offspring SVL, and litter size among litters produced by captive, enclosure, and free-living females (Table 2.2).

Manipulating the amount of supplemental food provided to females in enclosures did not result in females on the high-energy diet gaining significantly more weight prior to ovulation than females on the low-energy diet ($F_{1,16} = 2.16$, $p = 0.16$). Not surprisingly, there were no differences in offspring mass or offspring SVL between females on the low- and high-energy diets (Table 2.3). Females on the low-energy diet did produce larger litters than females on the high-energy diet. However, because females in better condition produce larger litters (see above), this result may be due to females on the low-energy diet being in better condition in the spring than females on the high-energy diet ($F_{1,16} = 4.35$, $p = 0.05$). After controlling for maternal condition, litter size no longer differed between females on the low- and high-energy diets ($F_{1,14} = 1.71$, $p = 0.21$). There were no differences in variance in offspring mass, offspring SVL, or litter size between females on the low- and high-energy diets (Table 2.3).

I examined whether the mean or variance in offspring size or litter size differed among females' litters for free-living females captured in different years. There were no differences in offspring mass or length among years, although there was a tendency for mass to differ among years ($p = 0.07$, Table 2.4). Mean litter sizes were significantly smaller in 1997 and 1998 than in 1994 and 1995 (Table 2.4). Litter sizes were also

smaller in 1997 than in 1996. There were no differences in the variances of offspring mass, offspring SVL, and litter size among years (Table 2.4). I investigated whether mean annual temperatures during the period when follicles were developing (May to mid-June) could explain the differences in offspring size and litter size among years. Across years, mean annual temperature during this period ranged between 13.4 to 16.7 °C. There was a significant difference in mean temperature among years ($F_{4, 225} = 4.8$, $p = 0.001$); 1998 was significantly warmer than 1994 and 1997. Despite differences in temperature and offspring/litter size among years, there was no correspondence between temperature variation and offspring size variation.

Within Litter Variation

I used partial correlation to examine whether maternal size, maternal condition, litter size, and timing of birth influenced within-litter variation in offspring size. Partial correlation was necessary because many of these maternal attributes were correlated. I removed the effect of litter size to examine the effects of maternal size and condition, I removed the effect of maternal condition to examine the effect of birth date, and I removed the effect of maternal size to examine the effect of litter size. I had predicted that variation within litters would increase with date of birth. Within-litter variation in offspring SVL, but not mass, increased with date of birth (Table 2.5). Contrary to my expectations, within-litter variation did not increase with litter size (Table 2.5). Within-litter variation did not vary with maternal size or condition (Table 2.5)

I found some evidence that food availability influenced within-litter variation in offspring size. I had predicted that within-litter variation in offspring size would increase

with maternal weight gain during pregnancy (Table 2.5). Within-litter variation in offspring mass, but not SVL increased with maternal weight gain. Contrary to my expectation that captive females and females on the high-energy diet would have higher within-litter variation, within-litter variation in offspring mass and SVL was significantly higher for enclosure females than for captive and free-living females. There was no difference in within-litter variation between captive and free-living females (Table 2.2). Within-litter variation in offspring mass and SVL also did not differ between enclosure females on high- and low-energy diets (Table 2.3). I also found no differences in within-litter variation in offspring mass and SVL across five years (1994 - 1998) for free-living females (Table 2.4).

Finally, I examined how consistent individual females were in their reproductive output across their litters to determine whether within-population variability in offspring size was due mainly to differences among females. Over the course of the study, 15 females produced more than one litter (11 females had 2 litters, 3 females had 3 litters, and 1 female had 4 litters). After controlling for maternal size using ANCOVA, individual females were consistent in producing offspring that were similar in mass and SVL across their litters. In addition, females showed consistency in the amount of variation in offspring mass and SVL across their litters (Table 2.6). Females were not consistent in the number of offspring that they produced across their litters (Table 2.6). Using reduced major axis regression, females were consistent in producing offspring that were similar in mass (Table 2.6, Fig. 2.5.) and SVL (Table 2.6, Fig. 2.6), and that had a similar amount of variation in mass and SVL. Females were not consistent in the number of offspring they produced across their litters (Table 2.6).

I investigated these results further by examining whether time between successive litters influenced how consistent females were in their reproductive output. I predicted that females would be more consistent when their litters were produced closer in time. Using ANCOVA, I found that females whose litters were separated by one or two years were consistent in producing offspring of similar size and with similar variation in size (Table 2.7). Again, females were not consistent in the number of offspring they produced in each litter. Females whose litters were separated by three or four years were not consistent in their reproductive output (Table 2.7). Using regression analysis, I found a significant positive relationship between the time between litters and how dissimilar offspring were in mass ($r = 0.60$, $n = 15$, $p = 0.02$, Fig. 2.7). An unexpected result was that similarity in litter size increased with the time between litters ($r = -0.57$, $n = 15$, $p = 0.02$, Fig. 2.8.). I found no significant relationships between the time between litters and how dissimilar offspring were in SVL ($r = 0.44$, $n = 15$, $p = 0.10$), in variation in mass ($r = 0.05$, $n = 15$, $p = 0.85$), and in variation in SVL ($r = 0.21$, $n = 15$, $p = 0.45$).

Maternal influences on sex ratio variation

Overall, data on offspring sex ratios were obtained from 117 litters (Table 2.8) composed of 1049 males and 1116 females. The overall sex ratio of 0.48 males to 0.52 females did not differ from equality ($X^2 = 2.07$, $df = 1$, $p = 0.15$).

I reconsidered whether maternal size, offspring size, or timing of birth were associated with sex ratio. Contrary to Weatherhead et al.'s (1998) analysis, larger mothers did not produce proportionately more daughters ($r = 0.27$, $n = 34$, $p = 0.12$). In addition, I did not find that litters with larger offspring were disproportionately female ($r = 0.14$, $n =$

34, $p = 0.43$). I examined whether the disagreement between the results from my analysis and those of Weatherhead et al. (1999) was due to the inclusion of enclosure females in my analysis. After removing enclosure females from my analysis, there remained no relationship between maternal size and sex ratio ($r = 0.33$, $n = 16$, $p = 0.21$) and no relation between offspring size and sex ratio ($r = -0.16$, $n = 16$, $p = 0.55$). Thus, it is unclear why results from the two studies differed.

Similar to Weatherhead et al. (1998), I did not find that date of birth had a significant effect on sex ratio ($r = -0.04$, $n = 117$, $p = 0.68$) I also examined whether females in better condition, because they give birth earlier (results above), produced litters that were disproportionately female. However, I found no relationship between maternal condition and sex ratio ($r = -0.11$, $n = 89$, $p = 0.29$).

I examined whether sex ratio influenced relative tail length in males and females. I had expected that tail length of females would increase with an increase in the proportion of males in litters, whereas tail length of males would decrease with an increase in the proportion of females in litters. Overall, the range in sex ratio among the litters varied between highly female-biased to highly male-biased (Table 2.8). Mean tail length of males (5.67 ± 0.42 S.D.) was significantly longer than that of females (4.96 ± 0.37 S.D., $t = 45.37$, $n = 103$, $p < 0.001$). Relative tail lengths of females did not increase significantly with the proportion of males ($r = 0.14$, $n = 103$, $p = 0.17$) and relative tail lengths of males did not decrease significantly as the proportion of females increased ($r = 0.12$, $n = 103$, $p = 0.24$). Thus, there was no evidence to suggest that sex ratio influences tail length in female or male water snakes.

Discussion

Variation in Offspring Size and Litter Size

Among litter variation

My results are consistent with a previous study of water snakes and those of several other snakes demonstrating that offspring size is subject to maternal effects (Seigel and Ford 1992; Weatherhead et al. 1999). The patterns of variation in offspring size generally correspond to those found by Weatherhead et al. (1999) for the same population of water snakes. In general, larger litters were composed of smaller offspring. At a given litter size, larger females produced larger offspring. In contrast to Weatherhead et al. (1999) who found no effect of birth date on offspring size, I found that larger offspring were born earlier. In addition, I did not find that larger litters were born earlier. For offspring size, the difference in results appears to be due to the inclusion of enclosure females in the analysis. For litter size, the inclusion of enclosure females does not explain the difference in results. Weatherhead et al. (1999) suggested that their result that larger litters were born earlier was largely a consequence of captive females giving birth earlier and to larger litters than free-living females. I found that litter sizes of captive females in my study were smaller than those in Weatherhead et al.'s (1999) study. This difference may be due to females in Weatherhead et al.'s (1999) study investing energy gained prior to ovulation into increasing the size of their litters, whereas females in my study did not invest this energy into their litters (see below).

Weatherhead et al. (1999) found some evidence that offspring size and litter size increased with the amount of weight females gained in the spring prior to ovulation. There was no evidence that females in my study invested energy gained prior to ovulation

into their litters. In my study, snakes began mating earlier than those used in Weatherhead et al.'s (1999) analysis (unpubl. data). Consequently, timing of ovulation may have been earlier for females in my study, which would have limited the amount of energy females gained prior to ovulation and thus what was available to invest in their litters. Gregory and Skebo (1998) found no evidence that female garter snakes (*Thamnophis elegans*) invested energy gained during pregnancy into their litters. Seigel and Ford (1992) found no effect of food intake during pregnancy on the size of offspring produced by *T. marcianus*, but females provided with more energy had larger litters. The occurrence of conflicting results within species and among closely related species suggests that more study is needed to determine the potential for females to invest energy gained prior to ovulation into their litters.

Litter size varied among free-living females across five years, but mean offspring size and variance in offspring size and litter size did not vary across years. Several other studies of snakes have found that litter size appears to be more plastic than offspring size (Andrén and Nilson 1983, Ford and Seigel 1989; Seigel and Ford 1991, 1992). Recently, Barron and Andraso (2001) showed that the number of follicles destined to be ovulated in the spring was almost twice as high, on average, for females on a high-energy diet the previous fall compared to females on a low-energy diet. Because food availability is likely to differ among years, differences in litter size among years may reflect annual differences in energy available to invest into litters.

Within-litter variation

My examination of the association between maternal phenotypes and within-litter variation in offspring size revealed that the amount of within-litter variation was associated with birth date. I had predicted that offspring born later in the season would be more variable because environmental uncertainty increases with timing of birth. Within-litter variability in offspring length, but not mass, increased with birth date. If late summer conditions are poor, shorter and fatter offspring should benefit from having larger energy stores. If late summer conditions are better and neonates have time to forage before hibernation, longer and thinner offspring may have an advantage because locomotory performance increases with body length (Arnold and Bennett 1988). In addition, other traits, such as head size, scale allometrically with body length (Forsman and Lindell 1993). Consequently, larger snakes may have higher foraging success because increased locomotory ability should increase capture success and larger head size allows larger snakes to consume a greater range of prey sizes (Shine 1991; Forsman and Lindell 1993).

This result suggests that females may have some control over how energy is allocated to growth versus storage in their offspring and that females might adaptively tradeoff length versus storage with their date of birth. This interpretation requires that females must be able to predict when they will give birth. I found that females in poor condition in the spring give birth later. Thus, females may use their condition in the spring to predict when they are going to give birth. The reason why females in poor condition give birth later is unknown, but there are several possibilities. Females in poor condition may take longer to acquire the energy needed for ovulation (Weatherhead et al.

1999) or emerge from hibernation later than females in better condition. It is also possible that, if males discriminate among females, females in poor condition may mate and ovulate later than females in better condition.

I found some evidence that food availability during follicular development influenced within-litter variation in offspring size. However, this was evident only when I examined the correlations between maternal weight gain and within-litter variation. The patterns of within-litter variation observed among captive, enclosure, and free-living females and between high-energy females and low-energy females provided no evidence that food intake influenced variation in offspring size within litters. The correlations revealed that within-litter variation in mass, but not length, increased with maternal weight gain prior to ovulation. In contrast, Seigel and Ford (1992) showed that within-litter variation in length, but not mass, was higher for females on a high-energy diet. My prediction that variation would increase with mass gain was based on the hypothesis that females may have less control over allocation as the amount of energy to allocate becomes larger. Although my results are consistent with this prediction, there is little information on how females provision embryos relative to one another. High within-litter variation could result from females investing differently in all offspring or investing similarly in most and very differently in a few. I had also predicted that within-litter variation would increase with litter size, based on a suggestion by Seigel and Ford (1992) that females with large litters may have less control over energy allocation to follicles. However, I found no correlation between litter size and within-litter variation. Again, information on how females provision ova relative to one another would provide a basis for interpreting this result.

Using data on females that gave birth more than once during the course of the study, I found that individual females were consistent in producing similar-sized offspring and offspring that had similar variation in size across their litters. Females were not consistent in the number of offspring produced across their litters, even though this analysis controlled for changes in female size between litters. Comparable results were found in water pythons (*Liasis fuscus*) (Madsen and Shine 1996) and smooth snakes (*Coronella austriaca*) (Luiselli et al. 1996). These results suggest that within-population variability in offspring size is a result of intrinsic differences among females (e.g., genetics), whereas variation in litter size is influenced more by proximate factors. However, an examination of how consistency varied with time between litters provided more insight into these relationships. I found that females producing litters separated by one or two years were consistent in producing offspring of similar size and that had similar variation in size, but females producing litters separated by three or four years were not consistent. These results suggest that proximate factors, rather than intrinsic differences among females, account for consistency in offspring size. Litter size was not consistent regardless of the time between litters. This result may suggest that litter size is strongly affected by even small differences in proximate factors among years. Regression analyses of consistency versus time between litters revealed that offspring were more dissimilar in size as time between litters increased. However, I found no relationship between the time between a female's litters and the consistency of variance in offspring size. Unexpectedly, I found that females produced litters that were more similar in size as the time between litters increased. A potential explanation for this result may be that females who breed infrequently produce litters that are less different in size. In my

analysis only a female's first and last litters were used. For females that produced litters separated by three or four years, I examined whether these females bred less frequently. Most females produced at least one litter between the litters used in the analysis. Thus, breeding frequency does not appear to explain the increased consistency in litter size with time, and an explanation for this result remains unclear.

Sex ratio variation

In contrast to the results of Weatherhead et al. (1998), I found little evidence that females adjust sex ratios adaptively, even after removing data on enclosure females which were not used in Weatherhead et al.'s (1998) study. The reason for the difference between studies is unclear. Low sample sizes used in this study (34 vs. 83) may be one reason for these differences. However, litter sex ratios have not been shown to vary consistently with maternal characteristics in other species. In two studies investigating how litter sex ratios varied with maternal size in the garter snake, *Thamnophis sirtalis*, one study showed no variation between maternal size and sex ratio (Sommerer 2000), whereas the other showed that the proportion of males increased with maternal size (Dunlap and Lang 1990). Clearly, more information is needed on how sex ratios vary with maternal characteristics in a number of snake species before considering whether variation is adaptive.

I also found no evidence that litter sex ratios influence the expression of tail length in males and females. Osypka and Arnold (2000) found that tail length (measured as counts of subcaudal scales) of female *T. elegans* was more feminized in male-biased litters, but only in one of the two populations they examined. Other studies have found

that the position of males relative to females *in utero* influences the expression of sexually dimorphic traits (e.g., Gandelmann et al. 1977). These studies indicate that females that are surrounded only by males *in utero* exhibit more masculine traits, compared to females surrounded only by other females. While these effects remain to be tested in snakes, my results did not support the prediction that the overall litter sex ratio affected the expression of a sexually dimorphic trait in water snakes.

Conclusions

My study provides evidence that maternal characteristics account for variation in offspring size within and among litters in *N. sipedon*. Female water snakes that produce larger litters have smaller offspring. At a given litter size, larger females produce larger offspring. This study adds to the growing number of studies demonstrating that the idea of an optimal offspring size cannot account for widespread variation in offspring size within populations (Bernardo 1996b). Understanding whether variation in offspring size is adaptive will require information on the reproductive physiology of energy allocation in snakes and on the effect of size on offspring fitness. Few studies have investigated the effect of size on survival of neonate and juvenile snakes, due to the difficulty in obtaining data on survival under natural conditions (Parker and Plummer 1987). In Chapter Four, I examine whether variation in offspring size translates into variation in fitness using data on survival of snakes from birth to one year measured under semi-natural conditions.

Table 2.1. Mean ($\pm$ S.E.) measurements for northern water snake females, offspring, and litters in the captive, enclosure, and free-living samples. Measurements of females were made in the spring. Condition is ln-transformed mass relative to ln-transformed snout-to-vent length (SVL). Means for offspring measurements are means of litter means. Sex ratios are means of the proportion of males in each litter. Free-living females were not measured in the spring in 1997-1999. Means of maternal characteristics presented for "all" free-living females are based on individuals captured in 1994-1996 (37 individuals).

	n	Females			Offspring			Litters	
		Mass (g)	SVL (cm)	Condition	Mass (g)	SVL (cm)	Size	Mass (g)	Sex ratio
Captive	33	284.6 (12.4)	73.5 (0.9)	0.03 (0.03)	4.1 (0.1)	16.8 (0.2)	18.9 (1.0)	75.3 (3.7)	0.50
Enclosure									
High energy	10	272.3 (16.1)	75.4 (1.6)	-0.08 (0.03)	4.3 (0.5)	16.5 (0.3)	16.4 (2.2)	63.8 (8.5)	0.52
Low energy	8	336.8 (32.5)	78.3 (3.0)	0.02 (0.03)	3.8 (0.2)	16.1 (0.5)	24.6 (3.3)	90.2 (12.0)	0.44
All	18	300.9 (18.1)	76.6 (1.6)	-0.03 (0.03)	4.1 (0.1)	16.4 (0.3)	20.1 (2.1)	75.5 (7.6)	0.48
Free-living									
1994	11	246.0 (15.7)	70.6 (1.3)	-0.004 (0.03)	4.0 (0.1)	17.0 (0.2)	19.2 (1.0)	77.6 (5.2)	0.49

1995	8	275.7 (25.6)	74.2 (1.8)	0.002 (0.05)	3.9 (0.1)	16.5 (0.2)	21.3 (1.4)	79.8 (4.5)	0.53
1996	18	241.7 (9.4)	70.3 (0.7)	-0.01 (0.02)	4.5 (0.1)	17.1 (0.2)	16.9 (1.0)	74.5 (4.2)	0.43
1997	8	-	-	-	4.3 (0.2)	16.9 (0.2)	19.5 (2.0)	81.6 (7.3)	0.47
1998	5	-	-	-	4.4 (0.1)	17.3 (0.1)	18.2 (3.1)	79.9 (13.2)	0.46
1999	2	223.5 (0.5)	70.1 (1.8)	-0.06 (0.07)	4.2 (0.6)	17.0 (0.9)	14.0 (2.0)	56.0 (1.0)	0.66
All	52	249.6 8.4	71.2 0.7	-0.005 0.02	4.2 (0.1)	17.0 (0.1)	18.5 (0.6)	76.9 (2.6)	0.48

Table 2.2. Variability in offspring size and litter size among captive, enclosure, and free-living female northern water snakes. Means ($\pm$ S.E.) for offspring mass and SVL are means of litter means. Coefficients of variation (CV) were calculated for each litter and the mean percent variation is presented. Mean CVs of offspring mass and offspring SVL are adjusted for maternal size and litter size. Mean CVs of litter size are adjusted for maternal size. F-statistics are based on transformed data when assumptions of parametric tests were not met. Means with the same letter designation are not significantly different.

Rearing Condition	Captive	Enclosure	Free-living		
N	33	18	52	F	p
Offspring mass (g)					
Mean	4.1 $\pm$ 0.1	4.0 $\pm$ 0.1	4.3 $\pm$ 0.1	2.77	0.07
Among-litter CV	10.0%	10.3%	9.4%	0.07	0.93
Within-litter CV	6.1% ^A	8.5% ^B	4.8% ^A	5.28	0.01
Offspring SVL (cm)					
Mean	16.7 $\pm$ 0.1 ^A	16.2 $\pm$ 0.2 ^B	17.0 $\pm$ 0.1 ^A	7.18	0.001
Among-litter CV	4.1%	4.4%	3.4%	0.75	0.47
Within-litter CV	2.3% ^A	3.1% ^B	2.1% ^A	3.58	0.03
Litter size					
Mean	18.7 $\pm$ 0.8	17.7 $\pm$ 1.2	19.4 $\pm$ 0.7	0.73	0.48
Among-litter CV	21.7%	30.2%	21.8%	1.48	0.23

Table 2.3. Variability in offspring size and litter size between female northern water snakes in enclosures on high and low energy diets. Means ($\pm$ S.E.) for offspring mass and SVL are means of litter means. Coefficients of variation (CV) were calculated for each litter and the mean percent variation is presented. Mean CVs of offspring mass and offspring SVL are adjusted for maternal size and litter size. Mean CVs of litter size are adjusted for maternal size. F-statistics are based on transformed data when assumptions of parametric tests were not met.

Diet	Low Energy	High Energy		
N	8	10	F	p
Offspring mass (g)				
Mean	4.0 $\pm$ 0.2	4.2 $\pm$ 0.2	0.61	0.45
Among-litter CV	12.2%	6.4%	1.11	0.31
Within-litter CV	7.2%	9.7%	0.45	0.51
Offspring SVL (cm)				
Mean	16.3 $\pm$ 0.5	16.4 $\pm$ 0.4	0.01	0.94
Among-litter CV	6.8%	2.8%	2.58	0.13
Within-litter CV	2.6%	3.6%	0.99	0.35
Litter size				
Mean	23.7 $\pm$ 2.2	17.1 $\pm$ 2.0	4.83	0.04
Among-litter CV	30.6%	26.4%	0.18	0.68

Table 2.4. Variability in offspring size and litter size among free-living female northern water snakes in 1994-1998. Mean $\pm$ SE for offspring mass and SVL are means of litter means. Coefficients of variation (CV) were calculated for each litter and the mean percent variation is presented. Mean CVs for offspring mass and offspring SVL are adjusted for maternal size and litter size. Mean CVs of litter size are adjusted for maternal size. F-statistics are based on transformed data when assumptions of parametric tests were not met. Means with the same letter designation are not significantly different.

Year	1994	1995	1996	1997	1998	F			p
N									
Offspring mass (g)									
Mean	4.1 ± 0.1	4.0 ± 0.2	4.5 ± 0.1	4.1 ± 0.2	4.3 ± 0.2	2.33		0.07	
Among-litter CV	7.9%	7.3%	9.0%	8.9%	5.0%	0.51		0.73	
Within-litter CV	5.6%	5.1%	4.3%	4.3%	4.6%	0.89		0.48	
Offspring SVL (cm)									
Mean	17.2 ± 0.2	16.7 ± 0.2	17.1 ± 0.2	16.6 ± 0.3	17.1 ± 0.3	1.25		0.30	
Among-litter CV	2.3%	3.1%	3.7%	3.4%	1.4%	1.25		0.30	
Within-litter CV	2.5%	2.2%	1.9%	1.9%	1.9%	1.47		0.23	
Litter size									
Mean	20.3 ± 0.9 ^A	20.2 ± 1.1 ^A	19.1 ± 0.8 ^{AC}	15.4 ± 1.2 ^B	16.3 ± 1.4 ^{BC}	3.66		0.01	
Among-litter CV	14.0%	14.1%	22.4%	19.2%	33.2%	1.69		0.17	

Table 2.5. Spearman rank correlations between within-litter variation and maternal SVL, maternal condition, litter size, date of birth, and maternal weight gain during the period when follicles were developing (May-June). Correlations for maternal size, maternal condition, litter size, and date of birth are partial correlations (see text for explanation).

	n	Within-litter variation in mass		Within-litter variation in SVL	
		r	p	r	p
Maternal SVL (cm)	89	0.05	0.63	0.04	0.68
Maternal condition	89	0.07	0.51	0.02	0.83
Litter size	103	0.08	0.44	0.05	0.65
Date of birth	103	0.07	0.49	0.26	0.02.
Maternal weight gain	57	0.30	0.02	0.21	0.11

Table 2.6. Consistency in reproductive output across successive litters of individual female northern water snakes. ANCOVA results indicate the effect of maternal identification on various reproductive traits after controlling for maternal size. Significant p values indicate that females were consistent in their reproductive output across their litters. Results from the reduced major axis regression analysis indicate whether there is a correspondence in reproductive traits between females' first and last litters after correcting reproductive traits for maternal size (residuals from regression of trait on maternal size). Confidence intervals (CI) are used to test whether slopes are significantly (*) different from zero. A significant positive slope indicates that females are consistent in their reproductive output across their litters. F-statistics are based on transformed data when assumptions of parametric tests were not met.

Offspring Trait	ANCOVA			Regression Analysis	
	df	F	p	m	95% CI
Mass (g)	14, 19	4.54	0.001	1.11	0.60, 1.63*
CV mass	14, 19	2.36	0.04	2.08	1.03, 3.13*
SVL (cm)	14, 19	4.15	0.002	1.41	0.71, 2.11*
CV SVL	14, 19	4.20	0.002	2.37	1.20, 3.55*
Litter size	14, 19	1.15	0.38	-1.63	-2.59, -0.66*

Table 2.7. Consistency in reproductive output across successive litters of individual female northern water snakes in relation to time between their first and last litters.

ANCOVA results indicate the effect of mom identification on various reproductive traits after controlling for maternal size. Significant p values indicate that females were consistent in their reproductive output across their litters. Females were separated into two groups based on whether their litters were separated by one or two years or by three or four years. F-statistics are based on transformed data when assumptions of parametric tests were not met. When transformations did not allow data to meet assumptions of tests, a test statistic is not provided.

Offspring Trait	Litters 1-2 years apart			Litters 3-4 years apart		
	df	F	p	df	F	p
Mass (g)	8, 7	10.48	0.003	5, 5	0.75	0.62
CV mass	8, 7	6.63	0.01	5, 5	0.45	0.80
SVL (cm)	8, 7	10.70	0.003	5, 5	-	-
CV SVL	8, 7	3.95	0.04	5, 5	1.65	0.30
Litter size	8, 7	0.94	0.54	5, 5	0.80	0.60

Table 2.8. The number of litters, sex ratios, and birth dates (Julian date) of northern water snake litters produced by captive, enclosure, and free-living females

	Captive	Enclosure	Free-living	Overall
Litters	33	18	66	117
Percent male				
mean	50.2	48.2	47.9	48.6
range	26-85	31-75	13-75	13-75
Birth date				
mean	228.5	245.1	245.2	240.5
range	213-247	233-267	225-262	213-267

Figure 2.1. Litter size relative to maternal size in northern water snakes.

Figure 2.2. Mean offspring mass relative to litter size in northern water snakes.

Figure 2.3. Litter size relative to maternal condition in northern water snakes. Condition is estimated using the residuals from a regression of ln-transformed mass relative to ln-transformed SVL of females.

Figure 2.4. Birth date of litters relative to maternal condition in northern water snakes.

Figure 2.5. Reduced major axis regression of mean offspring mass of female northern water snakes' last litters versus mean offspring mass of their first litters. Offspring mass has been corrected for maternal SVL (see methods).

Figure 2.6. Reduced major axis regression of mean offspring SVL of female northern water snakes' last litters versus mean offspring SVL of their first litters. Offspring SVL has been corrected for maternal SVL (see methods).

Figure 2.7. Relationship between the time between an individual female northern water snakes' first and last litters and the difference in offspring mass between these litters. Difference in mass between litters is the absolute difference in mass corrected for maternal SVL (see methods).

Figure 2.8. Relationship between the time between an individual female northern water snakes' first and last litters and the difference in litter size between these litters.

Difference in litter size between litters is the absolute difference in litter size corrected for maternal SVL (see methods).

CHAPTER THREE
THE EFFECT OF MATERNAL THERMOREGULATORY BEHAVIOUR ON
OFFSPRING PHENOTYPES IN NORTHERN WATER SNAKES, *NERODIA*
SIPEDON

Introduction

Environmental conditions experienced during embryonic development can have profound effects on the phenotypes of reptiles. The most notable example occurs in some oviparous species in which offspring sex is determined by the ambient temperatures to which eggs are exposed in nests (Bull 1980). Ambient temperature also affects other characteristics of neonatal reptiles, including timing of birth (Shine and Harlow 1993, 1996), body size and shape (e.g., Burger 1989, 1990, 1991; Shine and Harlow 1993), thermal preference (Blouin-Demers et al. 2000), locomotory performance (e.g., Shine and Harlow 1993), anti-predator behaviour (Burger 1990, 1998a,b), and growth rate (e.g., Brana and Ji 2000; Wapstra 2000). Female reptiles have some potential to mediate the effects of ambient temperature on the phenotypes of their offspring. In oviparous species, females can choose among nest sites or control when oviposition takes place (Shine and Harlow 1996; Shine et al. 1997b). In viviparous species, females can use behavioural thermoregulation to regulate their own body temperature and thus, the temperatures at which their offspring develop (Gibson and Falls 1975; Schwarzkopf and Shine 1991; Sorci and Clobert 1997; Wapstra 2000). Consequently, some of the variation in phenotypes among neonatal reptiles arises through a female's influence on the thermal conditions that her offspring experience during development. Hence, this type of maternal control also allows for the possibility

of adaptive manipulation of offspring phenotypes (Shine and Harlow 1993; Sorci and Clobert 1997; Shine and Downes 1999; Swain and Jones 2000). In this chapter I explore the consequences of altering the opportunities for thermoregulation available to gravid female northern water snakes (*Nerodia sipedon*) on a variety of neonatal characteristics likely to affect neonatal, and thus maternal, fitness.

Northern water snakes are viviparous and thus females have the potential to control the thermal environment their embryos experience during development. Several studies of viviparous reptiles have shown that gravid females maintain body temperatures that differ (i.e., are higher or lower) from non-gravid females and males (e.g., Beuchat 1986; Blazquez 1995). Brown and Weatherhead (2000) recently demonstrated that female northern water snakes thermoregulate more carefully and maintain higher body temperatures when gravid. Maintenance of higher body temperatures by gravid females could reflect a strategy to decrease developmental time of offspring (Beuchat 1986). This would benefit females by reducing the period they are gravid and thus potentially more vulnerable to predation, and would benefit offspring by allowing them a head start on growth prior to entering hibernation. Alternatively, an increase in preferred body temperature could reflect an optimum temperature for development of other offspring characteristics. The ability of gravid females to maintain their preferred body temperature could be limited by thermal constraints if the thermal environment does not allow females to achieve their preferred body temperature. Brown and Weatherhead (2000) found that water snakes do not fully exploit opportunities to maintain preferred body temperatures and suggested that this might reflect ecological constraints such as foraging or avoiding predation. My first objective is to determine

how manipulation of maternal thermoregulatory opportunities affects the phenotypes of neonatal water snakes. I consider two alternative hypotheses. First, the greater the opportunity females have to maintain their body temperatures in their preferred range, the more likely it is that offspring will have characteristics that should be favourable for survival (i.e., “warmer is better”). Second, the more closely thermoregulatory opportunities mimic patterns experienced by females in the wild, the more likely it is that offspring will have characteristics that should be favourable for survival (i.e., “natural is better”). I consider several neonatal attributes that may influence survival of offspring, including timing of birth, body size, condition, locomotory performance, and defensive behaviour.

The biological relevance of environmentally induced phenotypes ultimately depends on whether these phenotypes persist long enough to affect the fitness of individuals (Elphick and Shine 1998). Several studies have shown that some effects may persist for at least several months (e.g., Shine and Harlow 1993; Elphick and Shine 1995; Burger 1998a,b), whereas others have shown that effects may diminish, change, and emerge some time after birth (Elphick and Shine 1995). Thus, my second objective is to investigate whether any effects of maternal basking opportunity on offspring phenotypes persist, change, or emerge through time. I compare phenotypes of offspring measured at birth with those measured a second time just prior to hibernation. Because northern water snakes in the population I studied enter hibernation in as little as a few weeks after they are born and hibernate for approximately six months (Weatherhead et al. 1999), the phenotypes of offspring entering hibernation seem likely to affect their probability of survival over winter.

Materials and Methods

I collected adult females (>55 cm) in 1999 from several marshes near the Queen's University Biological Station (QUBS) in eastern Ontario, Canada (45° 37' N, 76° 13' W). These females were collected at two different periods during the active season and were maintained in captivity until they gave birth. I used three different treatments designed to bracket the thermal conditions gravid female northern water snakes experience in the wild during gestation (mid June through August). Brown and Weatherhead (2000) showed that gravid females in the wild maintain body temperatures within their preferred range (25-30 °C) from mid morning through early evening (8-12 h per day). During the night females remain as warm as their environment allows, which is generally just below their preferred temperature (20-25 °C). In the first treatment, females were captured from the wild in late April and early May. These females were provided with an electric heating rock for 24 h per day. Females in this treatment were mated in captivity by housing them with several males. The mean ($\pm$ S.E.) temperature of the room where females were housed was 24.4 ± 0.4 °C. Thus, this treatment provided females with thermal conditions superior to those experienced in the wild, in that they could achieve preferred body temperatures throughout the entire day.

A second group of females was collected in mid June, after mating in the wild was completed. These females were randomly divided into two groups and provided with an electric heating rock for either 4 or 8 hours per day. Females in the 4 and 8 hour treatment groups were maintained in the same room with a mean ($\pm$ S.E.) temperature of 22.9 ± 0.3 °C. Thus, the 8-hour treatment provided females with thermoregulatory opportunities similar to what they would experience in the wild, while the 4-hour

treatment provided females with conditions inferior to what they would experience in the wild. Although females were collected at different times during the active season, gestation begins after female water snakes ovulate in mid to late June (Bauman and Metter 1977, Feaver 1977, Aldridge 1982). Thus, temperature effects on developing offspring can only have an effect during this time. Because all females were captured by mid-June and temperature treatments were started in mid-June, females in all treatment groups were exposed to their particular thermal regime when gestation began.

Because my goal is not only to assess how gestation temperature regimes affect neonatal phenotypes, but also to relate those effects to the natural situation, I also use data on offspring from females that completed most of gestation in the wild. From 1994-1999 I collected sexually mature females from the wild (hereafter “free-living females”) in late July to late August and brought them into the lab to give birth (see Chapter One). By including free-living females from six years, my intention was to determine both the mean and the range of conditions experienced in the wild for comparison with the experimental females and their litters.

Females were housed in fibreglass tanks (74 cm x 59 cm x 55 cm) or plastic containers (65 cm x 55 cm x 55 cm) lined with artificial grass matting. All females were provided with a large water dish to allow them to submerge. Females were fed fresh or previously frozen minnows one or two times per week. Females in the 4- and 8-hour basking treatments were fed after heating sources were removed for the day so that feeding did not interfere with thermoregulation.

Beginning in August, all females were monitored closely so that data could be collected from their offspring shortly after birth. I measured several characteristics of

litters and individual offspring that were likely to influence offspring survival, including timing of birth, body size, condition, locomotory performance, ventral scale anomalies, defensive behaviour and growth rate. I assumed that the probability of survival should be higher for offspring that were born earlier, larger, in better condition, and were more defensive, and that had fewer scale anomalies, higher locomotory performance, and faster growth rates. Female water snakes give birth in late summer or early fall (Weatherhead et al. 1999). Consequently, offspring have little time to forage before they enter hibernation. Thus, offspring that are born earlier should have more time to forage and store energy prior to hibernation. In addition, offspring that are born larger, in better condition, and that grow faster, should have higher survival because they enter hibernation with larger energy reserves or because their size and condition may make them more successful in foraging prior to hibernation. Ventral scale anomalies have been shown to be associated with skeletal anomalies such as fused vertebrae or duplicated ribs (Osgood 1978; Merila et al. 1992; Forsman et al. 1994) and locomotory performance has been shown to decrease with increasing numbers of anomalies (Arnold and Bennett 1988). Locomotory performance should influence foraging success and the ability to escape from predators. There is also empirical evidence that some of these variables influence survival of neonatal reptiles (e.g., timing of birth: Olsson and Shine 1997; size: Ferguson and Fox 1984; Jayne and Bennett 1990; Janzen 1993; locomotory performance: Arnold and Bennett 1988; ventral scale anomalies: Osgood 1978; Arnold and Bennett 1988; Merila et al. 1992; Forsman et al. 1994).

Within 24 hours of birth, defensive behaviour of neonates was tested. These tests were conducted at the time neonates were removed from their mothers' housing

container. To conduct a trial I waited until a neonate had moved away from its mother and siblings. I then simply reached into the tank approaching each individual “head on” and recorded whether or not it responded, where a response was either flight or a strike at my hand. I then calculated the proportion of neonates within each litter that responded.

After removal from their housing containers, neonates were weighed and measured within 48 hours after birth. Partly-developed offspring and follicles were not included in measures of offspring size but were included in measures of litter size. Fully-developed, stillborn offspring were included in measures of offspring and litter size. I measured condition of neonates by taking the residuals from a regression of ln-transformed mass on ln-transformed snout-vent length (SVL). I recorded the number of ventral scale anomalies (e.g., insertion of half ventrals) on each individual and calculated the percentage of offspring in each litter with anomalies. Swimming speed was measured by “chasing” neonates the length of a trough of water 2 m long and ca. 14 cm wide. Water temperature in the trough was maintained at 25 °C. A stopwatch was used to record the time it took each individual to swim the length of the trough. Each individual was tested twice and its burst speed calculated using the faster of the two trials.

After offspring were measured and tested, they were marked by inserting a passive integrated transponder (PIT) tag (Anitech Identification Systems, Markham, Ontario) or by clipping a unique number of ventral scales. Offspring were then released into outdoor enclosures (see Chapter One) or were released at the site of capture of their mothers. In early October, prior to neonates entering hibernation, I recaptured neonates

from the enclosures and measured their SVL, mass, swimming speed, and calculated their condition and growth in SVL and mass. After offspring were measured, they were released into artificial hibernacula within the enclosures.

Statistical analysis

I used ANOVA to compare maternal characteristics, birth dates, and proportion of offspring that were stillborn/deformed, had scale anomalies, and that responded defensively among the treatment groups. Data involving proportions were arcsine transformed prior to analysis to normalize data. To compare all other offspring phenotypes measured at birth, I used nested ANOVA with basking treatment and litter number nested within basking treatment as the factors. To compare phenotypes of offspring measured prior to hibernation, I used the same nested ANOVA as above and included the number of days since birth as a covariate in the analysis. Because these two sets of analyses (phenotypes at birth and days prior to hibernation) involved the same individuals, these tests were not independent. Thus, I also performed repeated measures ANCOVA using the design above to determine whether there was an overall effect of basking regime on offspring phenotypes.

Analyses were carried out using SAS (SAS 1999) software. Data were transformed when they failed to meet assumptions of tests. Significance was assessed at $\alpha = 0.05$.

Results

I measured traits of offspring from eight females in the 4-hour treatment, six females in the 8-hour treatment, seven females in the 24-hour treatment, and 49 free-

living females. Because maternal size and litter size influence offspring phenotypes (e.g., size, see Chapter Two), I examined whether there were differences among basking treatments in the size of females or in the size of their litters which could confound interpretation of my results. Female size and litter size did not differ across basking regimes (maternal size: $F_{3,23} = 1.14$, $p = 0.35$; litter size: $F_{3,66} = 1.58$, $p = 0.20$) so these variables were not controlled for in subsequent analyses.

Timing of birth

Because rate of development of reptilian embryos is temperature dependent, I expected that females given more opportunity to bask would give birth earlier. As expected, basking regime had a significant effect on timing of birth ($F_{3,66} = 18.33$, $p < 0.0001$, Fig. 3.1). Females given unlimited opportunity to bask (24 hours) gave birth significantly earlier than free-living females and females allowed to bask for 4 or 8 hours. Although females allowed to bask for 8 hours gave birth, on average, 6 days earlier than females allowed to bask for 4 hours, this difference was not significant. My goal in allowing one group of females to bask for 8 hours per day was to provide these females with thermoregulatory opportunities similar to those available to females in the wild. Timing of birth did not differ between free-living females and females allowed to bask for 8 hours, suggesting that the 8-hour regime provided thermoregulatory opportunities similar to those in the wild. Females allowed to bask for 4 hours gave birth later than free-living females, as expected, because females in this group experienced thermoregulatory opportunities inferior to those available in the wild.

Offspring characteristics at birth

Overall, I found a significant effect of basking regime on all phenotypes (Table 3.1). Females allowed to bask for 24 hours produced the longest neonates, females allowed to bask for 8 hours produced the shortest neonates, with the other two groups being intermediate (Fig. 3.2a). The superior performance of the 24-hour females supports the hypothesis that females able to maintain their preferred temperature for longer would produce offspring with traits favourable to survival. However, the poor performance of the 8-hour females, particularly in comparison to the 4-hour females, is not consistent with this hypothesis. Similarly, the poor performance of the 8-hour females is also inconsistent with the hypothesis that females provided with thermoregulatory opportunities similar to those available in the wild would produce superior offspring.

The differences in mass among offspring were similar to differences in length (Fig. 3.2b). Females allowed to bask for 24 hours produced offspring that were significantly heavier than free-living females and females allowed to bask for 8 hours. However, mass was similar among offspring from females allowed to bask for 4 and 24 hours. Again, these results do not support the hypotheses that females with more opportunity to bask or females provided with basking opportunities similar to those in the wild would produce offspring with traits favourable to survival.

Females allowed to bask for 4 hours produced offspring that were in the best condition, followed by offspring from free-living females, with offspring from the other two treatments being in the poorest condition (Fig. 3.2c). The superior performance of the 4-hour females is clearly inconsistent with both the hypotheses being tested.

Free-living females produced offspring that swam the fastest, followed by offspring from females that were allowed to bask for 24 and 4 hours, with offspring from the 8-hour females swimming the slowest (Fig. 3.2d). Offspring length is significantly positively related to swimming speed ($r = 0.29$, $n = 622$, $p < 0.0001$). Thus, differences in swimming speed among treatment groups might be due to differences in length rather than to an effect of maternal environment. After controlling for length, using residuals from a regression of swimming speed on SVL, offspring from free-living females were still significantly faster than those from the other treatment groups, followed by offspring from 4-h females, then offspring from 24-h females and 8-h females (Fig. 3.2e). The superior performance of free-living females and the poor performance of 8-hour females again meant that neither of the two hypotheses under consideration was supported.

Litter characteristics at birth

I found a marginal effect of basking regime on the percentage of deformed and/or stillborn offspring in a litter ($F_{3, 66} = 2.58$, $p = 0.06$). The percentage of deformed and/or stillborn offspring was lowest for offspring from free-living females (2.5%), followed by those from females allowed to bask for 4 hours (5.3%), 24 hours (8.3%), and 8 hours (14.9%). The low percentage of deformed and/or stillborn offspring from females allowed to bask for 4 hours did not support the hypothesis that females with more opportunity to maintain their preferred temperature would have characteristics favourable to survival. The high occurrence of deformities/stillbirths for females in the

8-hour treatment was inconsistent with the hypothesis that natural thermal regimes are superior.

Basking regime had a significant effect on the percentage of ventral scale anomalies in litters ($F_{3, 30} = 5.19$, $p = 0.005$, Fig. 3.3). The percentage of anomalies was lowest for offspring from females allowed to bask for 24 hours, followed by those from free-living females, females allowed to bask for 8 hours, and those allowed to bask for 4 hours. Because anomalies decreased with the time females were allowed to bask, this result supports the hypothesis that females with more opportunity to maintain their preferred temperature produce offspring with characteristics favourable to survival.

There was little variability in the percentage of snakes that responded defensively across basking regimes, resulting in defensive behaviour not varying significantly among basking regimes ($F_{3, 32} = 1.71$, $p = 0.18$). Consequently, there was no support for the hypotheses that females with more opportunity to maintain their preferred temperature or those with thermoregulatory opportunities similar to the wild would have characteristics favourable to survival.

Offspring characteristics at hibernation

I measured offspring on a second occasion 14-62 days after birth, just prior to them entering hibernation. Using these measurements, I examined whether the effects of basking regime on offspring phenotypes observed at birth persisted or changed prior to hibernation, or whether effects emerged prior to hibernation. After controlling for differences among individuals in the time since their date of birth, I found a significant effect of basking treatment on all phenotypes (Table 3.2). However, the patterns of

differences among groups at hibernation for a given phenotype were generally not consistent with differences observed at birth (comparison of Figs. 3.2a-e with Figs. 3.4a-e). For example, for offspring SVL, the initial size advantage of offspring from females allowed to bask for 24 hours compared to those from free-living females diminished prior to hibernation. However, the initial size advantage of offspring from females allowed to bask for 4 hours persisted when compared to offspring from females allowed to bask for 8 hours. These results indicate that the effect of basking treatment may have long-term effects on some offspring phenotypes, but these effects may change over time.

Measuring offspring prior to hibernation also allowed an investigation of how basking regime influenced growth of offspring. After controlling for differences among individuals in the number of days since birth by using days since birth as a covariate in the analysis, I found that offspring from free-living females grew more in length than offspring from females allowed to bask for 4, 8, and 24 hours (Fig. 3.4f). Growth in length is correlated with length at birth ($r_s = -0.12$, $n = 272$, $p = 0.05$), so I removed any confounding effects of length at birth on growth using initial SVL as a covariate in the analysis. After doing so, growth of offspring from free-living females remained higher than that of offspring from any other group. In addition, offspring from females allowed to bask for 4 hours grew more than those from females allowed to bask for 8 hours (Fig 3.4g). Because offspring from free-living females grew faster than offspring from any other group, these results do not support the hypotheses that females with more opportunity to maintain their preferred temperatures would have characteristics favourable to survival. The superior performance of free-living females is consistent

with the hypothesis that naturally occurring thermal conditions produce superior offspring, but support for that hypothesis is weakened by the poorer performance of offspring from females in the 8-h treatment.

Most offspring lost weight prior to hibernation. Generally, loss in mass was similar for offspring from all groups, but offspring from free-living females lost less weight than offspring from females in the 4-h treatment (Fig. 3.4h). Growth in mass is also correlated with mass at birth ($r_s = -0.38$, $n = 272$, $p < 0.0001$). After controlling for mass at birth, offspring from free-living females lost less weight than offspring from females allowed to bask for 4 or 8 hours (Fig. 3.4i). Because loss in mass tended not to differ among basking regimes, there was no support for the hypotheses that females with more opportunity to maintain their preferred temperature or those with thermoregulatory opportunities similar to the wild would have characteristics favourable to survival.

Effect of timing of birth on offspring phenotypes

Earlier birth of offspring is assumed to enhance offspring survival because offspring born earlier have a head start on growth prior to entering hibernation. Because timing of birth differed among some of the treatment groups, I was able to assess the effect of timing of birth on offspring phenotypes using measurements of offspring phenotypes made prior to hibernation. There was a significant effect of basking regime on all phenotypes at hibernation when this analysis was performed without controlling for differences among individuals in timing of birth (Table 3.2). Despite the earlier birth of offspring from females allowed to bask for 24 hours, SVL prior to hibernation of

offspring from this treatment was similar to that for offspring from free-living females and those from females allowed to bask for 4 hours (Fig 3.5a). This result is surprising considering that offspring from females allowed to bask for 24 hours started with the double advantage of being both larger and born earlier than offspring from any other group. Mass and condition of offspring from females allowed to bask for only 4 hours (and thus born later than offspring from other groups) was greater prior to hibernation than that of offspring of any other group (Figs. 3.5b, c). These results are less surprising given that both mass and condition were relatively high at birth for offspring from females allowed to bask for 4 hours. Absolute and relative speed did not appear to be influenced by timing of birth (Figs. 3.5d, e). The differences in speed among groups observed at birth were generally similar prior to hibernation. Despite a longer period over which to grow, offspring from females allowed to bask for 24 hours grew absolutely less in length than offspring from free-living females (Fig. 3.5f). Relative to their body size, offspring from females allowed to bask for 24 hours grew less compared to offspring from free-living females, more compared offspring from females allowed to bask for 8 hours, and had similar growth compared to offspring from females allowed to bask for 4 hours (Fig. 3.5g). For mass, offspring from females allowed to bask for 24 hours typically lost absolutely and relatively more weight than offspring born later (Figs. 3.5h, i)

Because analyses of offspring phenotypes at birth and prior to hibernation used measurements from the same individuals, these tests were not independent. Thus, I conducted repeated measures ANCOVA to determine whether basking treatment had an overall effect on offspring phenotypes. These analyses indicated that basking treatment

had significant effect on all phenotypes measured (SVL: $F_{3,242} = 25.16$, $p < 0.0001$, mass: $F_{3,242} = 63.37$, $p < 0.0001$; condition: $F_{3,242} = 47.88$, $p < 0.0001$; speed $F_{3,242} = 40.41$, $p < 0.0001$; relative speed: $F_{3,242} = 31.40$, $p = 0.01$). Thus, my results showing differences in offspring phenotypes among basking regimes measured at birth and prior to hibernation were not an artifact of these tests not being independent.

Treatment effects vs. litter effects

An advantage of using the nested ANOVA is that it allows an examination of the effect of differences among females (e.g., due to genetics) on variation in offspring phenotypes, as well as an examination of the effect of basking regime on offspring phenotypes. For all phenotypes, the effect of basking regime and the effect of litter identification nested within basking regime was significant. A significant effect of the nested factor indicates that siblings were more similar in phenotype than individuals from different litters. This effect was apparent using measurements at birth (Table 3.1) and those prior to hibernation (Table 3.2). Thus, variation in the phenotypes I examined were attributable to a combination of genetic effects and effects due to the maternal thermal environment.

Discussion

My results are generally similar to previous studies that demonstrate that the thermal environment available to gravid female reptiles influences the phenotypes of their offspring (e.g., Shine and Harlow 1993; Sorci and Clobert 1997; Shine and Downes 1999; Wapstra 2000). An interesting result of this study is that there was no

basking regime that produced offspring that were “optimal” in all phenotypes considered. Consequently, phenotypes assumed to be favourable to survival occurred with others assumed to be less favourable for survival. For example, females provided with superior basking conditions (24 hours) produced offspring that were born earlier and that were longer, but that were in poorer condition and grew in length more slowly. Similar results were found by Shine and Downes (1999) for offspring from female scincid lizards (*Pseudemoia pagenstecheri*) exposed to different prey and thermal environments during gestation. They suggested that tradeoffs between phenotypes prevent females from producing offspring that have an overall “optimal” phenotype. Thus, for water snakes, the benefits of earlier birth and/or large size presumably outweigh the costs of reduced locomotory performance and condition (but see below).

Collectively, my results provide little support for either the warmer-is-better or the natural-is-better hypotheses of maternal thermoregulation. For some phenotypes, females allowed to bask for 24 hours had phenotypes that were favourable to survival, providing support for the hypothesis that females allowed to maintain preferred temperature for longer would have characteristics favourable to survival. However, for comparisons between females allowed to bask for 4 and 8 hours, offspring from females allowed to bask for 8 hours typically displayed phenotypes less favourable to survival, even though they were allowed to maintain their preferred temperature for longer. In fact, offspring from females allowed to bask for 8 hours generally had phenotypes less favourable to survival compared to offspring from any other group.

In testing the hypothesis that natural thermoregulatory opportunities would produce superior offspring, I assumed that females with 8 hours to bask should be most

similar to females in the wild. Timing of birth did not differ between free-living females and those allowed to bask for 8 hours, which suggested my assumption was correct. However, phenotypes of offspring from free-living females and those from females allowed to bask for 8 hours differed in almost every other trait examined. Thus, the 8-hour regime appears not to have mimicked natural thermoregulation. A possible explanation for this difference may be that the range of variation around the preferred body temperature differs between females in the wild and my experimental treatment. Shine and Harlow (1996) showed that both mean and variance of incubation temperature influenced phenotypes of hatchling skinks. Despite the differences in phenotypes of offspring from free-living females and those in the 8-hour treatment, phenotypes of offspring from free-living females did not exhibit phenotypes that were always considered more favourable to survival. Consequently, results from this group also did not provide general support for the hypothesis that “natural is better”. Because no group exhibited an optimal combination of phenotypes, it appears that simple hypotheses about how temperature effects offspring phenotypes cannot be made for water snakes.

My results suggest that individual phenotypes appear to be sensitive to differences in temperature. While these results support the conclusion that no overall temperature regime can optimise all phenotypes simultaneously, they do not preclude the possibility that females optimise some offspring phenotypes, albeit at the expense of others. It seems likely that variation in some traits affect offspring survival more than others. At present nothing is known about the relative fitness consequences of different offspring traits. However, if natural selection has shaped embryonic development so

that the most important traits are optimised under natural thermoregulatory conditions, then traits optimised in offspring of free-living females should be most important. From this reasoning, I would predict that locomotory performance at birth should have a great impact on fitness. If locomotory performance is associated with increased foraging success, this may explain why offspring produced by free-living females lost less mass prior to hibernation than the offspring produced by females in the other basking regimes.

Consistent with the results of several other studies, I found that temperature effects during gestation could be detected several weeks after birth (e.g., Burger 1989; Shine and Harlow 1993, 1996; Shine et al. 1997b). However, I found that the patterns of differences among the groups for a given phenotype changed through time. Shine et al. (1997b) examined persistence of effects of incubation temperature on water python (*Liasis fuscus*) eggs incubated at three different temperature regimes measured at birth, 20 days after hatching, and 30 days after hatching. They found that the patterns of effects of incubation conditions at birth persisted for at least 30 days after hatching for some traits, whereas patterns changed or emerged for other traits. Some of the differences in patterns found in this study are due to differences in growth among basking regimes. For example, for SVL, the pattern of differences among treatment groups changed from birth to hibernation such that offspring from free-living females were similar in SVL at hibernation to offspring from females allowed to bask for 24 hours and became greater in SVL from those allowed to bask for 4 hours. A comparison of growth in SVL of offspring among the different basking regimes indicates that offspring from free-living females grew absolutely and relatively more in SVL than any

of the experimental regimes, whereas growth in SVL typically did not differ among offspring from any of the experimental regimes.

My examination of the effect of timing of birth on offspring phenotypes revealed a surprising result that offspring born the earliest did not retain their initial size advantage in length, nor did they gain an advantage in mass or condition. It is generally assumed that earlier born offspring have a survival advantage because they can forage and store more energy prior to hibernation, and there is some empirical evidence that earlier born offspring have higher survival (e.g., Olsson and Shine 1997). The failure of offspring from females in the 24-hour regime to maintain a size advantage is due to their slow growth. Consequently, in the time between birth and hibernation, offspring born later typically were able to attain the same length as those from females allowed to bask for 24 hours. Because offspring from females allowed to bask for 24 hours were also longer at birth, their maintenance requirements may have been greater, resulting in slower growth. Offspring from females allowed to bask for 24 hours generally were in poorer condition at birth and were typically slower swimmers relative to their body length, which may have influenced their ability to forage and grow prior to hibernation.

My analysis using nested ANOVA revealed a significant effect of litter nested within basking treatment on all phenotypes, measured at birth and again prior to hibernation. These results indicate that siblings were more similar in phenotype than offspring from different litters. Although some of the variation explained by litter identification probably results from genetic differences or differences in food intake among females within treatments (Chapter Two), some of this variation is likely due to

females within treatments differing in the amount of time they spent basking (e.g., Shine et al. 1997b), which was not accounted for directly in this study.

Overall, my results suggest that female water snakes in the wild are not constrained by temperature to produce suboptimal phenotypes. At hibernation, offspring from free-living females were similar in phenotype or had phenotypes considered more favourable for survival than females provided with unlimited thermoregulatory opportunities, despite differences in timing of birth and initial size at birth. One caveat to this conclusion is that in years where the prevailing ecological conditions are unfavourable for growth following birth, the smaller, later born offspring of free-living females may have a lower probability of survival. The next step is to examine which phenotypes influence survival of neonatal snakes and under which environmental conditions are some traits more important than others.

Table 3.1. The effect of basking regime and litter identification number nested within basking regime on mean offspring characteristics of northern water snakes measured at birth.

Trait	Treatment Effect		Litter Effect	
	F _(2,*)	P	F _(**,*)	P
SVL (mm)	100.9	<0.0001	29.6	<0.0001
Mass (g)	135.2	<0.0001	40.7	<0.0001
Condition	84.9	<0.0001	17.6	<0.0001
Speed (m/s)	47.1	<0.0001	9.2	<0.0001
Relative speed	34.6	<0.0001	7.9	<0.0001
Growth in SVL (mm/day)	24.4	<0.0001	3.0	<0.0001
Growth in mass (g/day)	4.6	0.004	4.9	<0.0001
Relative growth in SVL	30.0	<0.0001	2.7	<0.0001
Relative growth in mass	5.2	0.002	2.8	<0.0001

*df error for treatment and litter effect: SVL, condition = 1190; mass = 1192; speed, relative speed = 586

**df model for litter effect: SVL, mass, condition = 66; speed, relative speed = 32

Table 3.2. The effect of basking regime and litter identification number nested within basking regime on mean offspring characteristics of northern water snakes measured prior to hibernation, controlled or not controlled for number of days since birth.

Trait	Treatment Effect		Litter Effect	
	F _(3,*)	P	F _(25,*)	P
<i>Effect of timing of birth removed</i>				
SVL (cm)	38.7	<0.0001	19.9	<0.0001
Mass (g)	50.7	<0.0001	15.2	<0.0001
Shape	37.4	<0.0001	5.5	<0.0001
Speed (m/s)	31.1	<0.0001	3.0	<0.0001
Relative Speed	24.4	<0.0001	3.3	<0.0001
Growth in SVL (cm)	34.0	<0.0001	3.0	<0.0001
Relative growth in SVL (cm)	40.3	<0.0001	3.3	<0.0001
Growth in mass (g)	3.72	0.01	4.5	<0.0001
Relative growth in mass (g)	3.86	0.01	3.0	<0.0001
<i>Effect of timing of birth considered</i>				
SVL (cm)	47.4	<0.0001	19.8	<0.0001
Mass (g)	53.1	<0.0001	15.9	<0.0001
Shape	56.8	<0.0001	7.2	<0.0001
Speed (m/s)	27.4	<0.0001	1.6	0.03
Relative Speed	19.9	<0.0001	1.9	0.008
Growth in SVL (cm)	34.0	<0.0001	3.5	<0.0001
Relative growth in SVL (cm)	40.2	<0.0001	3.7	<0.0001
Growth in mass (g)	6.2	<0.001	5.5	<0.0001
Relative growth in mass (g)	6.7	<0.001	4.1	<0.0001

*df error =243 timing of birth considered growth in SVL and mass; all other df = 242.

Figure 3.1. Mean ($\pm$ S.E.) date of birth for female northern water snakes in the different basking regimes. Means with the same letter designation are not significantly different.

Figure 3.2. Mean ($\pm$ S.E.) traits of offspring at birth produced by female northern water snakes in the different basking regimes. Means with the same letter designation are not significantly different.

Figure 3.3. Mean ($\pm$ S.E.) proportion of ventral scale anomalies in female northern water snakes' litters among the different basking regimes. Means with the same letter designation are not significantly different.

Figure 3.4. Mean ($\pm$ S.E.) traits of offspring at hibernation produced by female northern water snakes in the different basking regimes. Means are adjusted for date of birth. Means with the same letter designation are not significantly different.

Figure 3.4 continued. Mean ($\pm$ S.E.) traits of offspring at hibernation produced by female water snakes in the different basking regimes. Means are adjusted for date of birth. Means with same letter designations do not differ significantly.

Figure 3.5. Mean ($\pm$ S.E.) traits of offspring at hibernation produced by female northern water snakes in the different basking regimes. Means with the same letter designation are not significantly different.

Figure 3.5 continued. Mean ($\pm$ S.E.) traits of offspring at hibernation produced by female northern water snakes in the different basking regimes. Means with the same letter designation are not significantly different.

CHAPTER FOUR
FACTORS AFFECTING SURVIVAL OF NEONATAL NORTHERN WATER
SNAKES, *NERODIA SIPEDON*

Introduction

When testing hypotheses about the fitness consequences of particular behaviours, researchers ideally want to consider not only the number of young produced by different individuals, but also the fate of those offspring after they are born. In reality, however, logistical constraints nearly always limit observations to the number of young produced. This pattern is certainly true for research on snakes. The cryptic nature of snakes generally, and neonatal and juvenile snakes in particular (Parker and Plummer 1987), have left the reproductive behaviour of snakes relatively poorly studied. Those studies that have investigated factors affecting fitness of offspring in snakes have generally used behaviour or morphology at birth or hatching as indices of fitness, rather than directly measuring survival and reproductive success (e.g., Burger 1990, 1998a,b; Shine and Harlow 1996). Notable exceptions are studies by Jayne and Bennett (1990) and Charland (1989) that investigated how neonatal size affected over-winter survival in garter snakes (*Thamnophis sirtalis*) and rattlesnakes (*Crotalus viridis*), respectively. My goal in this chapter is to use patterns of survival of neonatal northern water snakes (*Nerodia sipedon*) to test hypotheses arising from the results presented in the preceding chapters.

Birth to one year of age appears to be a critical life-history stage for neonatal water snakes. For the population that I studied, indirect estimates of survival of neonates to one year of age ranged from 4-42% across six years, with a mean annual probability of

survival of 20% (Brown and Weatherhead 1999b). My goal here was to assess patterns of survival among individual neonates (e.g., relative to parentage), making it necessary to determine survival directly. Because neonates are difficult to study in the wild with available technology, I examined survival of neonatal water snakes in outdoor enclosures and artificial hibernation sites constructed within these enclosures. This approach restricted snakes to a relatively small area in which they could be located and observed, while providing a setting in which animals experienced most conditions present in the natural environment (e.g., variation in humidity, temperature, food) that could not easily be simulated in a laboratory setting.

In Chapter One, I found that larger male water snakes were more likely to sire at least some offspring in females' litters. This could have resulted from preferential mating by females, greater competitive ability of larger males, differential success of the sperm of males with which females had mated (e.g., Olsson et al. 1996b), or some combination of these factors. Regardless of the mechanism that produced this pattern, if it is advantageous to females, then one would predict that offspring of the most successful males should be more fit. Evidence from other taxa indicates that non-random mating by females can influence offspring fitness (e.g., insects: Partridge 1980, birds: Norris 1993, Moller 1994). In northern water snakes, larger males could sire more fit offspring if those offspring have phenotypic attributes that enhance survival (e.g., larger size). Alternatively, offspring sired by larger males could survive better, independent of any phenotypic attributes identified as enhancing survival. While both results could be consistent with those offspring being genetically superior, the latter result would more strongly suggest a genetic basis for enhanced survival. Size and age are strongly

correlated in water snakes (Brown and Weatherhead 1997), so larger males are also older. If survival ability is heritable, then older (larger) males may be genetically superior (e.g., Halliday 1978; Weatherhead 1984). Thus, my first objective is to determine whether females who mate with larger males enhance their fitness by producing neonates that survive better, and if so, whether their improved survival is a consequence of phenotypic attributes correlated with survival.

In Chapter One I reported that approximately 54% of females produce multiply sired litters. I investigated whether the presumed costs of multiple mating (e.g., time, energy, predation risk) might be compensated for by a reduction in either unfertilised ovules or stillborn young, but found no evidence of such benefits. However, I did find some evidence that multiple paternity increased phenotypic variance within litters, which could benefit females if having more variable offspring enhances mean offspring survival in unpredictable environments (Williams 1975). Here I consider the possibility that multiple mating enhances the genetic quality, and thus survival ability, of a female's offspring. Multiple mating also may provide females an opportunity to choose among sperm from different males ('cryptic female choice', Thornhill 1983; Eberhard 1996). Alternatively, multiple mating may promote intrauterine sperm competition, whereby ova are fertilized by the most competitive sperm, and thus presumably by the genetically superior male(s) (Curtisinger 1991; Madsen et al. 1992). In sand lizards (*Lacerta agilis*), Olsson et al. (1994b) found that the number of males with which a female mated was positively related with the hatching success of her eggs and with the proportion of her offspring that survived one year. In addition, the proportion of a female's offspring exhibiting malformations decreased with increasing number of mates of the female.

Similarly, Murie (1995) found that proportions of juvenile Columbian Ground Squirrels (*Spermophilus columbianus*) surviving to yearling age tended to increase with the number of mates of the mothers. My second objective is to compare over-winter survival between offspring from singly and multiply sired litters, and to determine whether differences in survival are attributable to phenotypic traits associated with survival ability.

Experiments designed to test predictions regarding the hypothesised advantages of multiple mating must deal with the problem of distinguishing between the number of males with which a female may have mated, and the number of males that sired young in the litter. Even if one could document all mating activity, which was not feasible in this study, other factors such as the amount of sperm contributed by each male would remain unknown. The approach I took in addressing this problem was twofold. First, I gave some females access to multiple males, but varied the number of males. This allowed me to compare offspring viability between singly and multiply sired litters from this group, as well as comparing offspring viability relative to the number of potential sires available. Second, I gave some females access to only a single male, ensuring their litters would be singly sired and that that outcome was also a result of the female only mating with one male.

The objectives outlined above require identification of phenotypic traits associated with survival of neonates, in order that the effect of those traits can be controlled when assessing possible genetic advantages of particular mating patterns. Thus, my final objective (although the first analytical step) in this study is to identify phenotypic attributes of neonates that affect their over-winter survival. Meeting this

objective is also relevant to other aspects of this study. In Chapter Two I showed that larger females produce bigger offspring, suggesting that females may be able to influence offspring viability independent of the paternity of the offspring. Body size has been proposed to be a major factor influencing fitness of neonatal snakes (Ferguson and Fox 1984; Charland 1989). Body size is correlated with several characteristics that might influence survival, including locomotory performance (Scribner and Weatherhead 1995; Chapter Three), endurance (Pough 1978), and the size of prey that can be eaten (Arnold 1983). In viviparous species living in temperate environments (such as northern water snakes), offspring are typically born in late summer or fall, shortly before hibernation. Consequently, newborn and juvenile snakes have little time to forage prior to hibernation and must rely on their energy reserves at birth. At temperate latitudes, neonates must endure long winters (ca. 6 months) at cold temperatures. Smaller neonates may be more susceptible to mortality during hibernation because they have smaller fat reserves (Whitford and Creusere 1977; Blem 1981; Gregory 1982). Few studies of snakes have attempted to address whether larger individuals have higher survival under natural conditions. In garter snakes (*Thamnophis sirtalis*), larger size at birth was associated with higher over-winter survival in one of two years (Jayne and Bennett 1990). In contrast, Charland (1989) found no effect of size at birth on over-winter survival of neonatal western rattlesnakes (*Crotalus viridis*).

Two factors that may influence the relationship between size at birth and survival are sex of offspring and timing of birth. Water snakes are sexually dimorphic in size, with females being the larger sex (Feaver 1977; Weatherhead et al. 1995), and this size dimorphism can be detected within litters at birth (Weatherhead et al. 1999). Thus, in my

analyses I assess whether sex influences survival. Timing of birth may also be important in determining over-winter survival, because offspring born earlier should have more time to forage and store energy before hibernation. Weatherhead et al. (1999) proposed that small offspring born earlier could survive as well as larger offspring born later because they have more time to forage before hibernation. However, in Chapter Three I showed that under semi-natural conditions, growth in the interval between birth and hibernation was minimal, even for offspring born early. Nonetheless, I also consider the effect of timing of birth when analysing survival.

In addition to assessing how size affects neonatal survival, I also consider two other attributes. Among adult water snakes in the same population, post-reproductive females with small fat stores have lower survival (Brown and Weatherhead 1997). Thus, I test the prediction that neonates in better condition are more likely to survive their first winter. I also assess the effect of neonatal locomotory performance on survival. The prediction here is less straightforward. More mobile neonates may be more likely to obtain a meal prior to hibernation, or find superior locations within the hibernation site, and thus be more likely to survive. Alternatively, excessive activity could deplete fat reserves and reduce neonatal viability.

Materials and Methods

Artificial hibernation sites

Six outdoor enclosures were constructed in 1986, each of which contained a hibernation site and a pond. All six enclosures were constructed in a field at the edge of a beaver pond near Queen's University Biological Station. Details of construction of

enclosures and associated ponds are presented in Chapter One. Each hibernation site (hereafter "den") was constructed by first excavating a hole approximately 1m deep. The adjacent beaver pond maintained a high water table that resulted in standing water at the bottom of the excavation. Cement blocks were stacked in each hole to enclose a space measuring approximately 40 x 40 x 50 cm. The top of the enclosed "box" was covered with a concrete paving stone (Fig. 4.1). The lower half of each den was loosely filled with hay, broken cement blocks, sticks, and plastic tubing to provide substrate for snakes to rest on and to move within the sites. An entrance to each den was constructed from a series of paired paving stones separated by steel reinforcing rods, creating a space approximately 4 cm in height leading from the interior to the exterior of the den (Fig. 4.1). Styrofoam sheets 5 cm thick were used to insulate the sides, top, and entrance of each den. Further insulation was provided by mounding topsoil over dens and den entrances. A thermocouple was placed in the center of each den with the lead running through the den entrance to the exterior. This allowed me to measure the internal temperature of dens over the winter.

Preliminary trials in 1996 revealed that neonates would enter the dens. However, neonates appeared to have a strong urge to disperse, resulting in them leaving the dens even as the weather deteriorated. To ensure that neonates remained in the dens, and to facilitate their capture when they emerged the following spring, I manufactured bags from fine nylon mesh that I used to enclose the entrance to each den. This allowed neonates to move in and out of the den entrance at will, but not to depart from the den. With the bags in place, some neonates often remained in the bags outside the dens in fair weather, but retreated inside the dens as the weather turned cold.

Measurement of survival

In late summer and early fall of 1997 to 1999, I put randomly chosen samples of neonates into outdoor enclosures. These neonates were born in captivity to females that were mated in captivity in the laboratory, in enclosures, or in the wild and brought into captivity after the mating season. Details of capture, processing of snakes, and housing conditions of females can be found in Chapters One to Three. I chose 10 offspring from each of 24 litters in 1997, 10-12 offspring from each of 23 litters in 1998 and four to 32 offspring from each of 29 litters in 1999 to be put in enclosures. In 1999 entire litters were placed in enclosures, whereas in 1997-1999 from the samples were portions of litters, randomly selected other than to have equal numbers of males and females whenever possible. All neonates were marked by inserting a PIT tag near the tail or by clipping a unique combination of ventral scales. All neonates from a litter were placed in the same enclosure. This was done to facilitate another study. Also, I assumed that because enclosures and dens were similarly constructed and located next to one another, conditions should not differ among dens. With the exception of one den site and enclosure, conditions appeared to be similar (see results). Across enclosures, the number of litters represented ranged from four to eight in any given year. Although the paternity of neonates was of central interest in this experiment, I did not determine the paternity of litters until after the field work was completed. Thus, samples were necessarily selected at random with regard to paternity.

In enclosures, neonates roamed freely around the enclosures and were free to forage in ponds within enclosures. Any feeding by these snakes relied on prey that

occurred naturally within the enclosures and minnows stocked in ponds (Chapter Two). Some snakes did grow in the period between being released and being recaptured prior to hibernation (Chapter Three), so food was available. Enclosures were constructed to deter predators from entering (Chapter Two), but were probably not "predator proof". In late September and early October just prior to when snakes should be entering hibernation in the wild, I attempted to recapture all individuals from enclosures by searching the enclosures and by scanning for PIT tags implanted in snakes. I searched each enclosure thoroughly for two weeks, so snakes not recaptured are assumed to have died. The sheet-metal fence surrounding each enclosure was buried in the ground and was ca. 75 cm high, which precluded escape by neonatal water snakes. Recaptured individuals were re-measured and then put into dens (in mesh bags in front of entrances to dens). In 1997 and 1999 temperatures inside each den were measured ca. once per month from October to April.

Beginning in mid March of the following year, mesh bags were checked daily to collect neonates that emerged from hibernation. The date of emergence of all snakes was recorded. Neonates were re-measured to document changes (e.g., mass loss) that occurred during hibernation. I continued checking the mesh bags until mid June, well after the last neonate had emerged. Neonates that did not emerge from hibernation were assumed to have died during hibernation. In 1998 and 1999, neonates that emerged were put in enclosures as part of another study on growth of juveniles and put in dens in the fall. In the spring of 2000, all neonates and juveniles emerging from hibernation were released at the sites of capture of their mothers.

Behavioural and morphological measures

I measured several characteristics of litters and individual offspring that could act as indices of offspring quality and/or that were likely to influence survival including mass, SVL, condition, and locomotory performance. Details of how these traits were measured are presented in Chapter Three.

Statistical Analysis

I examined factors influencing survival of offspring during two periods: between birth and hibernation and from the beginning to the end of hibernation. For my analysis of factors influencing survival of snakes between birth and hibernation I used data on snakes captured from enclosures to be put into hibernation. Three litters (47 individuals) were excluded from my analysis of pre-hibernation survival because these litters were born late in the season and were put directly into hibernation without first being put into enclosures.

For analyses involving measures of survival I performed analyses in two ways. I performed analyses using data on individuals without considering what litter they were from. In these analyses I used logistic regression with survived/did not survive (or recaptured/not recaptured prior to hibernation) as the binary response variable, and offspring traits as quantitative predictor variables. I also performed analyses using mean traits of individuals that survived and did not survive (or were recaptured or not) from individual litters. I used the general linear model procedure (PROC GLM) in SAS (SAS 1999) with the proportion of surviving (recaptured) offspring as the response variable and mean traits of offspring as predictor variables. In all but one case, the two methods

produced results that were qualitatively the same. Thus, I present results for my analyses based on individuals only.

To determine whether phenotypes and survival of offspring varied with the size of their fathers, I combined data on laboratory-mated and enclosure-mated females because I found a similar relationship between male size and mating success in both groups (Chapter One). I used PROC GLM with male size as a covariate and behavioural/morphological measures or proportion survived as the response variable (see results).

To determine whether offspring from multiply sired litters had higher survival, I used a frequency analysis with singly/multiply sired and survived/did not survive as factors. I also compared the proportion of offspring that survived between singly and multiply sired litters using PROC GLM with mating status (single or multiple) as the predictor variable and proportion of offspring surviving from litters as the response variable. In these analyses I included data on litters from the wild for which the minimum number of fathers siring litters had been determined using the method described in Chapter One.

Data on proportions of offspring surviving from litters were square root transformed so that data met assumptions of parametric tests. Nonparametric statistics were used when transformations failed to result in data that met assumptions of parametric tests. Additionally, all parametric analyses involving proportions of offspring surviving in litters were weighted by the number of snakes that were put into enclosures or into hibernation. Consequently, a litter in which ten snakes went into hibernation and survived (100% survival) was weighted twice as heavily as a litter in which five snakes

went into hibernation and survived (100% survival). Weighting in this way reduced the possibility that chance survival events would confound relationships in my data.

Similarly, analyses using mean traits of surviving/not surviving (or recaptured/not recaptured) offspring from litters were weighted by the number of individuals contributing to the means.

Analyses were carried out using SAS (SAS 1999). In all models, several other variables were included in order to control for effects of these variables that might have confounded my analyses (see below). I report only the Type III SS for the variables of interest. Significance of these terms was assessed at $\alpha = 0.05$.

Results

Between 1997 to 1999, I released a total of 950 neonatal water snakes into enclosures (Table 4.1). Of these, 638 (67.2%) neonates were recaptured in late September or early October and put into dens within enclosures. Of the snakes put into dens, 302 (47.3%) emerged in the springs of 1998-2000.

Pre-hibernation Survival

I examined whether attributes of neonates that were captured and put into hibernation differed from those that were not captured. The same attributes that could affect over-winter survival seem likely to influence the probability of survival before hibernation. Thus, I examined whether neonates that were larger, in better condition, and that had higher locomotory performance were more likely to be recaptured.

The number of snakes that were captured differed among years ($X^2 = 75.96$, $df = 2$, $p < 0.001$, Table 4.2) so year was included as a variable in my subsequent analyses.

The number of snakes that were captured also differed among enclosures ($X^2 = 19.37$, $df = 5$, $p = 0.002$, Table 4.3). Neonates in enclosure five were more likely to be captured. There were no differences in likelihood of capture in the remaining enclosures ($X^2 = 6.10$, $df = 4$, $p = 0.19$). Thus, data from enclosure five was omitted from my analyses. The probability of capture was higher for snakes that spent less time in enclosures ($\beta = 0.09$, $X^2 = 101.03$, $df = 1$, $p < 0.001$), so I controlled for this effect by including the date that snakes (or litters) were put in enclosures as a variable in my subsequent analyses.

Logistic regression revealed that neonates that were longer and heavier at birth had a significantly higher probability of capture (Table 4.4). There also was a trend for neonates in better condition at birth (mass relative to SVL) to have a higher probability of capture (Table 4.4). Neonates that were faster in locomotory trials also were more likely to be recaptured (Table 4.4). Because speed and body length are correlated (Chapter Three), I calculated speed of individuals relative to their SVL using residuals from a regression of speed on SVL. After controlling for SVL, faster neonates relative to their body length had a higher probability of recapture (Table 4.4). These results provide evidence that phenotypes at birth can influence survival of neonatal snakes prior to entering hibernation.

I also examined whether the proportion of offspring captured was higher for females who produced larger offspring or offspring in better condition, on average. The proportion of offspring captured did not vary with mean offspring SVL, mass, and condition (Table 4.5). Females that produced offspring that were faster on average had proportionally more of their offspring recaptured (Table 4.5). After controlling for body

size, females that produced offspring that were faster relative to their size also had proportionally more of their offspring recaptured (Table 4.5).

Over-winter Survival

I examined whether survival was similar across the years and across the six dens. I found significant differences in survival among years ($X^2 = 8.68$, $df = 2$, $p = 0.01$, Table 4.6). Annual differences in survival appear to be due to higher survival in 1999 compared to 1997 and 1998. Annual survival was 44.5%, 37.9% and 53.0%, in 1997, 1998, and 1999, respectively. There was no difference in survival between 1997 and 1998 ($X^2 = 1.34$, $df = 1$, $p = 0.25$). There also were significant differences in survival among dens ($X^2 = 18.35$, $df = 5$, $p = 0.003$, Table 4.7). Differences in survival among dens appears to be due to individuals in site two having particularly low survival. The remaining dens did not differ significantly in overall survival ($X^2 = 2.77$, $df = 4$, $p = 0.60$). Because of the differences in survival among hibernation sites and years, I eliminated data from site two and controlled for differences in survival among years by including year as a categorical variable in further analyses on survival (see methods). Annual differences in survival also provide an opportunity to assess whether the factors influencing fitness vary among years. In variable environments the relationship between survival and factors such as body size may vary among years (Ferguson and Fox 1984; Charland 1989). Thus, I included interaction terms between year and other independent variables in all analyses of survival to determine whether the factors affecting survival differed among years. However, in all analyses of offspring survival (below), the interaction term was not significant (all $p > 0.10$). Thus, there was no evidence that factors affecting offspring

survival differed among years. I removed the interaction term from all models and ran the reduced models. I report only results from these reduced models.

I examined whether differences among years and among hibernation sites were due to differences in ambient temperatures among years and among hibernation sites. There was a significant difference in temperature among years during the hibernation period (ca. 15 October to 31 March) ($F_{2, 431} = 3.69$, $p = 0.03$, Fig. 4.2). Mean air temp was significantly higher in the winter of 1999 than in the winter of 1998. Internal temperatures of hibernation sites did not differ in 1997 ($F_{5, 22} = 0.06$, $p = 0.99$) or in 1999 ($F_{3, 16} = 0.00$, $p = 0.99$) indicating that site two was not colder than any other site. Across den sites, the overall mean ($\pm$ S.E.) internal temperature of dens was 2.9 (± 0.7) °C in 1997 and was 4.3 (± 1.0) °C in 1999.

I captured snakes from enclosures over approximately two weeks and put snakes into hibernation after capture. The probability of survival was higher for snakes that entered hibernation later ($\beta = 0.06$, $X^2 = 8.17$, $p = 0.004$), so this factor was controlled for in subsequent analyses.

Offspring phenotypes

I examined whether neonates that were larger, in better condition, or had higher locomotory performance had higher over-winter survival. After controlling for differences in survival among years, logistic regression revealed that longer and heavier neonates had higher survival (Table 4.8). Neonates in better condition (mass relative to SVL) also had higher survival (Table 4.8). Neonates with higher locomotory performance also were more likely to survive over-winter (Table 4.8). After controlling for the effect

of SVL on speed, there was no effect of locomotory performance on probability of survival (Table 4.8).

I examined how mass of neonates changed during hibernation to determine whether the higher over-winter survival of larger neonates was due to them losing less mass during hibernation. After controlling for year and days in hibernation, heavier, larger neonates lost absolutely more mass during hibernation (SVL: $F_{1,214} = 6.00$, $p = 0.02$; SVL: $F_{1,214} = 12.58$, $p < 0.001$). However, there was no effect of size on the amount of mass lost relative to body size during hibernation (SVL: $F_{1,214} = 0.08$, $p = 0.78$; SVL: $F_{1,214} = 0.24$, $p = 0.62$). Thus, the enhanced survival ability of larger neonates was not due to them losing absolutely or relatively less mass during hibernation.

I also examined whether the proportion of offspring that survived was higher for females that produced offspring that were larger, in better condition, and had higher locomotory performance, on average. After controlling for differences in survival among years and mean date litters were put into hibernation, there were no relationships between mean litter SVL or condition and the proportion of offspring that survived in litters (Table 4.9). However, there was a tendency for litters of heavier neonates to have proportionally higher survival (Table 4.9). Females that produced litters of faster offspring, on average, had offspring with proportionally higher survival (Table 4.9). After controlling for the effect of body size on speed, females that produced relatively faster for their body size had offspring that tended to have higher survival (Table 4.9).

I examined whether female neonates have higher survival because they are significantly larger at birth. At birth, females were significantly larger in mass ($t = 3.83$, $n = 1497$, $p = 0.0001$), but not SVL ($t = 1.51$, $n = 1495$, $p = 0.13$). Mean mass ($\pm$ S.E) of

females was 4.33 g (± 0.02) and mean mass of males was 4.20 g (± 0.03). In addition, females were in significantly better condition than males ($t = 4.73$, $n = 1495$, $p < 0.0001$). Mean condition ($\pm$ S.E.) of females was 0.01 (± 0.003) and males was -0.01 (± 0.003). Despite differences in mass and condition, survival did not vary between the sexes ($X^2 = 0.99$, $df = 1$, $p = 0.32$).

Timing of birth could also influence survival of neonates because neonates born earlier have more time to forage prior to hibernation. Timing of birth may also have confounded my result that larger neonates have higher survival because larger neonates are born earlier (Chapter Two). Thus, higher survival could be due to larger neonates being born earlier, rather than an effect of size *per se*. However, logistic regression revealed that timing of birth did not have an effect on survival ($X^2 = 1.09$, $df = 1$, $p = 0.30$). Furthermore, females that produced litters earlier did not have offspring with proportionally higher survival ($F_{1, 50} = 0.11$, $p = 0.74$). Thus, timing of birth does not appear to have confounded my analysis of the effect of neonate size on survival.

Female mating behaviour

Non-random mating

I examined whether offspring sired by large males had higher survival than those sired by smaller males. The proportion of offspring that survived increased with the size of their fathers ($F_{1, 24} = 6.57$, $p = 0.02$, Fig. 4.3). Using the mean size of sires of females' litters, the proportion of offspring that survived tended to increase with the mean size of the males that sired a female's litter ($F_{1, 24} = 6.57$, $p = 0.06$). Thus, females mated to larger males receive indirect fitness benefits through increased viability of their offspring.

I examined whether increased survival of offspring of large males was due to offspring of larger males having traits more favourable to survival. There was no correlation between the size of a male and the size, condition, or locomotory performance of his offspring (Table 4.10). Thus, there was no indication based on behavioural or morphological measures that offspring of larger males had higher survival because they had traits favourable for survival.

Multiple mating

A frequency analysis of the number of individuals that survived from singly and multiply sired litters across years revealed that more offspring survived from multiply sired litters than expected ($X^2 = 4.19$, $df = 1$, $p = 0.04$, Table 4.11). Across years, ca. 36% of offspring from singly sired and 47% of offspring from multiply sired litters survived over winter. After controlling for date snakes entered hibernation and for differences in survival of offspring among years, the probability of a neonate from a multiply sired litter surviving over winter was 1.73 times greater than for a neonate from a singly sired litter ($\beta = 0.27$, $X^2 = 6.03$, $p = 0.01$).

I examined whether the proportion of offspring that survived was higher for females producing multiply sired litters than for females producing singly sired litters. After controlling for the differences in survival among years and mean date that offspring from a litter entered hibernation, there was no difference in the proportion of offspring surviving from singly and multiply sired litters ($F_{1,41} = 2.16$, $p = 0.15$). A possibility is that some females whose litters were sired by a single male had mated with several males and only one of the males had sired offspring. If so, combining these data with those

produced by females that had only mated with a single male might confound my examination of whether multiple mating enhances offspring quality. To examine this possibility, I used data on singly sired litters produced by females that were housed with a single male during the mating period. I had data on only four singly sired litters after excluding litters that were put into hibernation site two. Small sample sizes for this group resulted in data not meeting assumptions of parametric tests. I used a non-parametric Kruskal-Wallis test to examine differences between singly and multiply sired litters and controlled for year by including data only from 1997 and 1998 for which survival did not differ among years. I found that offspring from multiply sired litters had higher survival than offspring from singly sired litters ($X^2 = 5.87$, $df = 1$, $p = 0.02$, Fig. 4.4). These results provide evidence that females benefit by multiple mating through increased viability of their offspring. I examined whether these results were due to offspring from multiply sired litters having attributes favourable for survival. There were no differences in mean size, condition, or speed of offspring from singly or multiply sired litters (Table 4.12). Thus differences in survival do not result from offspring from multiply sired litters having characteristics more favourable for survival.

There was no relationship between the number of males housed with females during the mating season and the proportion of females' offspring to survive ($F_{1,37} = 0.06$, $p = 0.81$). There also was no difference between the proportion of offspring to survive in multiply sired litters sired by two or three males ($F_{1,17} = 0.72$, $p = 0.41$)

Discussion

In Chapter One, I documented that larger male water snakes were disproportionately successful at siring young, and that more than half of all female water snakes produced litters sired by more than one male. My goal in this chapter was to determine whether either of these phenomena had fitness consequences for females. Few similar studies have been conducted due to the difficulty in studying neonatal snakes under natural conditions (but see Olsson et al. 1994b, 1996a). The use of enclosures and artificial hibernation sites in this study allowed me to investigate fitness consequences of female mating behaviour by restricting neonates to an area in which their survival could be monitored.

I had two general objectives in this chapter. My first objective was to determine whether females producing young sired by larger males or multiple males increased their fitness through the higher survival of their offspring. My second objective was to attribute any effects on survival to the phenotypic or genotypic quality of offspring. In order to attribute effects on survival to phenotypic quality of offspring, required an understanding of the phenotypes that influence survival of neonatal water snakes.

Offspring phenotypes

A widely held view is that, when it comes to offspring, “bigger is better” (reviewed by Packard and Packard 1988). My results support this view. Larger neonates and neonates with larger energy stores relative to their body length were more likely to be recaptured prior to hibernation and to survive over winter. My results are generally consistent with the few other studies that have investigated the effect of body size and

condition on survival of neonatal snakes under natural or semi-natural conditions. Saint Girons and Naulleau (1981) found that for several viperid species, larger neonates had higher survival in outdoor enclosures during their first several months of life. Jayne and Bennett (1990) showed that larger neonatal garter snakes (*T. sirtalis*) had higher survival over winter. In contrast, Charland (1989) found that over-winter survival of neonatal rattlesnakes (*C. viridis*) was independent of mass and condition at birth. In comparison to neonatal garter and water snakes, neonatal rattlesnakes are considerably larger and heavier-bodied at birth. The relatively large size of neonatal rattlesnakes may have resulted in their survival over winter being independent of size at birth. In addition, over-winter survival was high (55%) in Charland's (1989) study. In years with lower survival, body size may be more important in determining which neonates survive (Charland 1989). Several studies of other reptiles have also shown enhanced survival of larger neonates prior to or during hibernation (e.g., Janzen 1993; Laurie and Brown 1990; Ferguson and Fox 1984).

One reason why smaller neonates are expected to have lower survival is because winter weight loss is typically greater in smaller animals (Gregory 1982). Proportionally, the amount of mass lost during hibernation did not vary with body size. Larger neonates lost absolutely more mass during hibernation, possibly because metabolic costs during hibernation are generally higher for larger individuals (Bennett 1982). Consequently, the amount of mass lost during hibernation does not appear to explain differences in survival among large and small neonates, unless the consequences for smaller neonates of losing the same proportion of mass as larger neonates differentially affects survival of small neonates. Dehydration during hibernation may contribute to mortality during hibernation

(Costanzo 1989; Prior and Shilton 1996). Larger neonates may be less prone to dehydration during hibernation because of their lower surface area to volume ratio (Charland 1989).

Locomotor performance was more important in determining whether individuals were recaptured prior to hibernation than whether or not they survived over winter. Under natural conditions, higher locomotor performance should make individuals more successful in avoiding predation and capturing prey (Miller et al. 1987). In my study, predators were excluded from enclosures so the benefit of increased performance on recapture prior to hibernation was unlikely to be associated with predator avoidance. Consequently, the most plausible explanation is that faster individuals were more likely to be recaptured because they were more successful in capturing prey and thus avoiding starvation prior to hibernation.

I did not explicitly examine whether timing of birth influenced survival prior to hibernation. However, the effect of timing of birth on pre-hibernation survival is reflected in the date that snakes were put in enclosures because snakes were put into enclosures shortly after they born. I showed that snakes that were put in enclosures later were more likely to be recaptured. Thus, timing of birth appears to have a negative effect on survival prior to hibernation, contrary to evidence in other reptiles suggesting that offspring born earlier have generally higher survival (Olsson and Shine 1997). In enclosures, most snakes lost mass prior to hibernation suggesting that snakes did not feed or fed little prior to hibernation. Whether this pattern is typical of neonatal water snake prior to hibernation under natural conditions is unknown, although fasting prior to hibernation is common in many reptiles (Gregory 1982). I also investigated whether timing of birth had an effect on

the probability of survival over winter but found no effect. This result is not surprising, however, because typically the only neonates that entered hibernation were those born late because survival of early born snakes was low in the pre-hibernation period. Consequently, there was little variation in timing of birth among individuals put into hibernation.

If individuals that are larger, in better condition, and that have higher locomotory performance have higher survival, it is expected that fitness would be higher for females producing litters of offspring that are larger, in better condition, and faster, on average. However, I did not always find that females that produced offspring with these characteristics had litters of offspring with proportionally higher survival. The most plausible reason for this inconsistency may be due to how these latter analyses were conducted. All individuals from a litter typically were not put into enclosures and/or did not enter hibernation. Thus, using mean traits of females' entire litters at birth in these analyses of survival may not have adequately represented the traits of neonates from litters that were used to measure survival. If so, analyses based on traits of individuals, rather than on mean traits of litters, would be more likely to reveal effects of phenotypes on survival.

Female mating behaviour

Non-random success of larger males

Several studies of snakes have shown that larger males have higher mating success (e.g., Madsen and Shine 1993; Shine et al. 2000). In spite of these observations, the fitness consequences of non-random mating in snakes are relatively unstudied (but

see Olsson et al. 1994b, 1996a). Here I have shown that females can acquire good genes for their offspring by mating non-randomly with larger males. Mean traits of offspring did not vary with the size of their fathers, suggesting a genetic rather than a phenotypic basis for enhanced survival of offspring of larger males.

Although I found that larger males have higher quality offspring, previous studies of this population of water snakes provide no evidence of selection on body size which would be expected if females prefer larger males or if larger males win mating competitions (Brown and Weatherhead 1999a; Prosser 1999). This would seem to indicate that body size is correlated with another trait that enhances offspring fitness (Howard et al. 1994). In water snakes, age and body size are positively correlated (Brown and Weatherhead 1999b). It has been suggested that females might prefer older males because viability selection should produce older males that are of higher genetic quality (Hansen and Price 1995; Beck and Powell 2000; Brooks and Kemp 2001). Female preferences for older mates have been documented in several taxa (birds: Weatherhead 1984; Kempenaers et al. 1997; mammals: Komers et al. 1997; insects: Jones et al. 2000). In addition, offspring of females mated to older males may be more fit, indicating that females may receive indirect fitness benefits by mating with older males (Olsson and Madsen 1995; Price and Hansen 1998; Jones et al. 2000). Future study should focus on determining whether older male water snakes are of higher quality by comparing fitness of offspring among males of known age while controlling for body size of males.

Multiple mating

Female water snakes commonly participate in several mating aggregations and produce litters sired by multiple males (Barry et al. 1992, Weatherhead et al. 1995; Prosser 1999, Chapter One). Because mating with several males has associated costs, the benefits of multiple mating presumably outweigh the costs. My results suggest that females receive indirect fitness benefits from multiple mating via increased survival of their offspring. Individual neonates from multiply sired litters were more likely to survive. In addition, proportionally more offspring survived from multiply than singly sired litters. However, I only found that offspring from multiply sired litters had proportionally higher survival than those from singly sired litters when I included singly sired litters produced by females that were housed with only a single male during the mating season. This result suggests that at least some females that were housed with more than one male during the mating season, but that had litters that were sired by a single male, may have mated with more than one male and only one male's sperm sired all offspring. I found no difference in mean traits of offspring between singly and multiply sired litters that could have accounted for offspring from multiply sired litters having higher survival.

If female water snakes can produce offspring with higher viability by mating with a single large male, why do they risk predation and expend time and energy to mate with several males? There are several possibilities. The simplest explanation may be that mating in large aggregations may limit females' control over their mating partners (Shine et al. 1999). If so, females may mate with a number of winners from aggregations to induce sperm competition to increase the possibility that their offspring are sired by the

highest quality male(s). Alternatively, females may mate with several males to increase genetic diversity of their litters. In Chapter One, I showed that the variance in phenotypic traits was higher in multiply sired litters. Consequently, females may mate with several males as a bet-hedging strategy because producing more variable offspring increases mean survival of offspring (Williams 1975). A final alternative is that genetic incompatibility between male and female gametes may make mating with a single male potentially risky to female fitness (Zeh and Zeh 1996, 1997; Jennions 1997). In sand lizards (*Lacerta agilis*) and adders (*Vipera berus*) offspring viability increases with the number of mates of the females (Madsen et al. 1992; Olsson et al. 1994b, 1996a; Olsson and Madsen 2001). This pattern is consistent with sperm competition resulting in the genetically superior male siring offspring. Recently, however, Olsson and Madsen (2001) demonstrated that this pattern most likely results from assortative fertilization with respect to genetic similarity between females and their mates such that offspring are sired by males less related to their mothers. In Chapter One I showed that the proportion of offspring sired in multiply sired did not vary with genetic similarity of parents at microsatellite loci. However, variation at other loci, particularly those that are not selectively neutral, may reveal phenomena not observed at mini- or microsatellite loci (Madsen et al. 2000; Olsson and Madsen 2001). Because it is unlikely that females could assess genetic compatibility of males prior to copulation (Zeh and Zeh 1997), a female water snake's best strategy would be to mate with large males from a number of aggregations, thus increasing the likelihood that her ova are fertilized by a genetically superior male(s) more compatible with herself.

Future research on mating behaviour of water snakes should focus on the mechanisms underlying why females mating with larger males and multiple males have offspring that are more fit. For non-random mating, future study should address whether any phenotypic characteristic correlated with male size (e.g., age) is associated with increased offspring fitness. For multiple mating, future study should focus on teasing apart whether increased phenotypic variance or genetic factors are associated with multiple mating. Comparisons of mean offspring survival between singly sired litters for which variance of offspring traits has been experimentally increased and multiply sired litters for which variance has been decreased would yield the most compelling evidence for females mating with multiple males to increase mean survival of their offspring. Recent advances in techniques to experimentally vary offspring size *in utero* would allow for such a study (e.g., Miles et al. 2000; Svensson and Sinervo 2000). Determining whether genetic compatibility between female water snakes and their mates explains why female water snakes multiply mate will require evidence for assortative fertilization with respect to genetic similarity revealed at loci other than microsatellite loci combined with evidence for differential survival of offspring from females mated with the same male.

Table 4.1. The number of neonatal northern water snakes that were put in enclosures and hibernation in 1997-1999 and that emerged from hibernation from 1998-2000.

Year	Enclosures	Hibernation	Emerged
1997	252	218	97
1998	230	116	44
1999	468	304	161

Table 4.2. Comparison of the number of neonatal northern water snakes that were captured or were not recaptured from enclosures in 1997-1999. Reported values are observed (and expected) numbers of neonates.

Year	Captured	Not Captured	Total
1997	218 (165)	34 (32)	252
1998	116 (151)	114 (79)	230
1999	257 (276)	164 (145)	421

Table 4.3. Comparison of the number of neonatal northern water snakes that were captured or were not captured from enclosures. Reported values are observed (and expected) numbers of neonates.

Enclosure	Captured	Not Captured	Total
1	140 (134)	64 (70)	204
2	108 (117)	70 (62)	178
3	50 (54)	32 (28)	82
4	53 (54)	29 (28)	82
5	144 (123)	44 (65)	188
6	96 (110)	73 (58)	169

Table 4.4. The probability of a neonatal water snake being recaptured prior to hibernation based on its phenotype.

Offspring trait	n	β	$X^2_{(df=1)}$	p
SVL (cm)	715	0.24	6.04	0.01
Mass (g)	715	0.39	7.44	0.01
Condition	715	0.47	3.24	0.07
Speed (m/s)	711	1.63	6.69	0.01
Relative speed	711	1.29	3.78	0.05

Table 4.5. The proportion of offspring to be recaptured from females' litters and the mean traits of offspring in litters.

Litter trait	β	S.E.	$F_{1,49}$	p
Mean SVL (cm)	0.03	0.02	2.29	0.14
Mean mass (g)	0.04	0.03	1.24	0.27
Mean condition	0.07	0.30	0.06	0.81
Mean speed (m/s)	0.58	0.17	12.33	0.001
Mean relative speed	0.66	0.19	11.71	0.001

Table 4.6. Comparison of numbers of neonatal northern water snakes that survived and did not survived in 1997-1999. Reported values are observed (and expected) numbers of neonates.

Year	Survived	Died	Total
1997	121 (114.8)	97 (103.2)	218
1998	72 (61.1)	44 (54.9)	116
1999	143 (160.1)	161 (143.9)	304

Table 4.7. Comparison of numbers of neonatal northern water snakes that survived and did not survive across hibernation sites. Reported values are observed (and expected) numbers of neonates.

Hibernation Site	Survived	Died	Total
1	75 (66)	65 (74)	140
2	39 (59)	85 (65)	124
3	21 (24)	29 (26)	50
4	25 (25)	28 (28)	53
5	76 (71)	73 (79)	149
6	66 (58)	56 (64)	122

Table 4.8. The probability of a neonatal water snake surviving over winter based on its phenotype.

Offspring trait	N	β	$X^2_{(df=1)}$	P
SVL (cm)	514	0.26	7.63	0.01
Mass (g)	514	0.52	11.95	0.001
Condition	514	0.69	6.23	0.01
Speed (m/s)	513	1.49	5.31	0.02
Relative speed	513	1.07	2.39	0.12

Table 4.9. The relationship between the proportion of offspring from females' litters to survive and mean traits of offspring in their litters.

Litter trait	β	S.E.	$F_{1, 50}$	P
Mean SVL (cm)	0.03	0.02	3.00	0.09
Mean mass (g)	0.06	0.03	3.64	0.06
Mean condition	0.30	0.30	0.99	0.32
Mean speed (m/s)	0.38	0.16	5.34	0.03
Mean relative speed	0.38	0.20	3.61	0.06

Table 4.10. Pearson Product-moment correlations between size of male water snakes and mean traits of their offspring.

Mean offspring trait	Correlation with male size	p
SVL (cm)	0.16	0.42
Mass (g)	0.04	0.84
Condition	-0.21	0.27
Speed (m/s)	-0.18	0.36
Relative speed	-0.27	0.15

Table 4.11. Comparison of the number of neonatal northern water snakes to survive over winter from singly and multiply sired litters. Reported values are observed (and expected) numbers of neonates.

	Survived	Died	Total
Singly sired	60 (70.5)	105 (126.5)	165
Multiply sired	94 (80.5)	106 (144.5)	200

Table 4.12. Comparison of mean ($\pm$ S.E.) traits of neonatal northern water snakes from singly and multiply sired litters. Singly sired litters are litters from females that were housed with only a single male during the mating season.

Litter Trait	Singly sired (n = 6)	Multiply sired (n = 21)	F_{1,23}	p
Mean SVL (cm)	17.3 (0.4)	16.7 (0.2)	0.92	0.35
Mean mass (g)	4.5 (0.2)	4.3 (0.1)	0.28	0.60
Mean condition	0.003 (0.01)	0.03 (0.02)	0.39	0.54
Mean speed (m/s)	0.64 (0.05)	0.60 (0.02)	0.57	0.46
Mean relative speed	0.01 (0.06)	0.002 (0.01)	0.05	0.83

Figure 4.1. Line drawing of an artificial den site.

Figure 4.2. Mean ($\pm$ S.E.) air temperatures during the period between 15 October to 31 March for 1997-1999.

Figure 4.3. The relationship between size of male water snakes and the proportion of their offspring to survive. Data are not corrected for other terms in the model (see results).

Figure 4.4. The mean ($\pm$ S.E.) proportion of neonatal northern water snakes to survive over winter from singly and multiply sired litters.

SUMMARY AND CONCLUSIONS

The purpose of this study was to identify ways that a female water snake could enhance the collective fitness of her offspring, and thus increase her own fitness. Specifically, I examined how female mating behaviour and maternal effects on offspring phenotypes might influence offspring fitness and measured fitness of offspring as their survival over winter in artificial hibernation sites.

In Chapter One, I found that larger males had higher reproductive success, particularly when the operational sex ratio (OSR) was male-biased. This result is consistent with my prediction that traits of males would be more important in determining male success when competition among males for access to females is intense. However, males are not able to forcibly copulate with females so females have some control over with which males they mate. Although it is unclear whether females preferentially mate with large males or whether large males win mating competitions, I proposed that the fitness consequences for offspring would be similar. If females preferentially mate with large males they presumably do so because large size indicates male quality and males that win competitions are presumably of higher quality than males that lose competitions.

In Chapter One, I also showed that approximately 54% of females produced litters sired by more than one male. In addition, larger females were more likely to produce multiply sired litters, supporting the hypothesis that males might prefer to mate with larger, more fecund females. I did not find any evidence that females engaged in multiple matings to insure that all of their ova were fertilized or to reduce the possibility of their offspring being sired by a related male. However, I did find that variance in phenotypic

traits was higher in multiply sired litters than in singly sired litters. This result provides some support for the hypothesis that females engage in multiple matings to increase genetic diversity of their litters, if mean survival of offspring is higher in more variable litters.

In Chapter Two, I showed that larger females produce larger offspring at a given litter size and females producing larger litters have smaller offspring. There was no evidence that maternal food intake influenced the mean or variance of offspring size among females' litters. However, females that gained more weight prior to ovulation produced litters of offspring that were more variable in size. I also examined how consistent individual females were in producing offspring of similar size across their litters. After controlling for the length of time between a female's litters, I found that females were consistent in producing offspring of similar size when their litters were separated by one or two years. However, females were not consistent in producing offspring of similar size when their litters were separated by three or four years. Females were generally not consistent in producing litters of similar size regardless of the time between litters. It appears that variation in offspring size and litter size results more from proximate factors than from genetic differences among females.

I found no evidence suggesting that females can influence the sex ratio of their litters. Females that produced large offspring and that gave birth early did not produce a disproportionate number of females. I also rejected the hypothesis that tail length of males would be shorter in litters where the sex ratio was female-biased and tail length of females would be longer in litters where the sex ratio was male-biased.

In Chapter Three, I showed that basking opportunity of females influenced the phenotypes and behaviour of their offspring. I rejected the hypothesis that the greater the opportunity females have to maintain their body temperature in their preferred range, the more likely it is that offspring will have characteristics that should be favourable for survival (i.e., “warmer is better”). I also rejected the hypothesis that the more closely thermoregulatory opportunities mimic patterns experienced by females in the wild, the more likely it is that offspring will have characteristics that should be favourable for survival (i.e., “natural is better”). No basking regime produced offspring with an overall ‘optimal’ phenotype, suggesting that temperature differentially affects the expression of traits or that females trade off some phenotypes for others. Some differences in phenotypes among offspring from the different basking regimes persisted until hibernation, suggesting that basking behaviour of females during gestation can have long-term effects on the phenotypes of their offspring.

In Chapter Four, I found that offspring that were larger, in better condition, and that had higher locomotor ability were more likely to be recaptured prior to hibernation and to survive over winter. I also found that females that produced litters sired by larger males and multiple males receive indirect fitness benefits through increased survival of their offspring. Previous studies do not provide evidence for selection on male body size, which would seem to indicate that body size is correlated with another trait that is associated with offspring fitness. It is unclear whether increased offspring survival for females that mate with multiple males results from increased phenotypic diversity of offspring or from genetic factors.

I suggest that future study should examine the mechanisms underlying why offspring produced by females that mated with larger males or that mated with multiple males are more fit. For non-random mating with respect to male size, the first step should be to determine whether any factor correlated with male size (e.g., age) is associated with enhanced viability of offspring. For multiple mating, future research should focus on determining whether increased phenotypic diversity or genetic factors are responsible for offspring in multiply sired litters having proportionally higher survival.

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