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SEXUAL SIZE DIMORPHISM
OF
NORTHERN WATER SNAKES,
Nerodia sipedon

by
Frances Ellen Barry

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

Master of Science

Department of Biology

Carleton University

Ottawa, Ontario

November, 1991

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Studies and Research acceptance of this thesis

**"SEXUAL SIZE DIMORPHISM OF NORTHERN WATER SNAKES,
Nerodia sipedon"**

submitted by Frances Ellen Barry
in partial fulfillment of the requirements for
the degree of Master of Science

A handwritten signature in black ink, appearing to be 'ARG', followed by a long horizontal line.

Chair, Department of Biology

A handwritten signature in black ink, appearing to be 'D. J. ...', followed by a long horizontal line.

Supervisor

Carleton University 1991

ABSTRACT

Because sexual size dimorphism in snakes is only slightly influenced by allometry and phylogeny, adaptive forces may explain the female-biased size dimorphism in northern water snakes. While male competition favoured larger males, males in the best condition achieved more mating opportunities. Therefore, the need to maintain good condition may limit male size. The observation that most water snake broods were sired by more than one male suggests that multiple mating by females may reduce the importance of competition among males. Larger females produced more offspring with less reproductive effort per progeny than smaller females indicating that natural selection may favour large female body size. Although males and females appeared to have different vulnerability to predation throughout the summer, the influence of differential predation risk on sexual size dimorphism requires further examination. Overall, both natural and sexual selection appear to influence the relative body sizes of male and female water snakes.

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GENERAL INTRODUCTION

In many animal species, one sex is larger than the other. Throughout the animal kingdom, there is great variation among species in the direction and degree of the difference in size between males and females. Illustrating the two extremes are the male northern elephant seals (*Mirounga angustirostris*) that can attain a size almost eight times larger than the female (LeBoeuf and Reiter 1988) and male angler fish that are less than a quarter the size of the females (Gould 1983). Much work on sexual size dimorphism (SSD) has focussed on species in which males are the larger sex, the pattern exhibited by the majority of mammalian and avian species. However, the typical pattern observed in the majority of taxa other than mammals and birds is that females are larger than males. In snakes, the majority of species exhibit female-biased SSD with females ranging in size from two-thirds to twice the size of males (Fitch 1981). In this thesis, I investigate a number of hypotheses proposed to explain the evolution of sexual size differences by examining patterns of SSD in snakes species in general, and in the northern water snake (*Nerodia sipedon*) in particular.

Various hypotheses, invoking both adaptive and non-adaptive mechanisms, have been proposed to explain the patterns of SSD in animal species. One potential mechanism which is non-adaptive that may constrain the evolution of SSD is allometry or the relationship among species between body size and SSD. There is a general trend that males are larger, relative to females, in larger species. Thus, the size of a species may limit the range of dimorphism it exhibits.

Additionally, SSD may be an evolutionarily conservative trait. Examples of other such evolutionarily constrained traits are the reproductive biology and the diet of a species (Shine 1989). Thus, females of a particular species may be larger than males because females were the larger sex in the ancestors of the species. Such phylogenetic constraint is another non-adaptive mechanism influencing patterns of SSD in species. Much variation in life history traits between species in many taxa has been attributed to the effects of allometry, phylogeny or both (insects: Fairbairn 1990; mammals: Cheverud et al. 1985, Clutton-Brock 1985; reptiles: Berry and Shine 1980, Stearns 1984, Dunham and Miles 1985 and birds: Bjorklund 1990). The relative sizes of the males and females of a species may not be due to adaptation to current or recent environmental or ecological conditions but may be a result of constraints of allometry or adaptations by ancestral species. In Chapter One, I examine the influence of allometry and phylogeny on the patterns of SSD in snake species.

Of the hypotheses that involve adaptive explanations for SSD, sexual selection is the most extensively studied and most widely supported (Darwin 1871, Trivers 1972). Sexual selection is the primary explanation for male-biased size dimorphism in many taxa. Large male size is favoured due to the advantages it confers in intra-sexual competition for access to females. Comparative studies have tested the prediction that, if sexual selection is an important influence on male body size, then male-biased dimorphism should be greater in species where males have the opportunity to mate with many females and/or large body size is advantageous to

males in acquiring many matings. These studies have found that, across species, as the number of females in a breeding group increases, the larger the males are relative to the females (Payne 1979, Harcourt et al. 1981, Jehl and Murray 1986, Knapton 1988). Shine (1978) showed that, among snakes, the majority of species known to exhibit male combat also showed male-biased size dimorphism. Thus, a possible explanation for the female-biased size dimorphism observed in other snake species may be that there is not strong competition among males for access to females and therefore, no selection favouring traits such as increased body size. In Chapter Two, I investigate whether the female-biased size dimorphism of northern water snakes is a consequence of either limited competition among males for mating opportunities or of sexual selection favouring a trait other than large body size in males.

Patterns of SSD in many species may be influenced by the mating system exhibited by the species (Shine 1980). For species in which a few males monopolize the females and achieve most of the matings, there is a high variance in male reproductive success. The higher the variance in male mating success, the more intense is the sexual selection operating on the males (Wade 1979, Wade and Arnold 1980). In some species, females are able to influence this variance in mating success between males. If females mate with multiple males, then the competition among males will be reduced along with the selection favouring large males. In Chapter Three, I investigate the possibility that female northern water snakes mate

with more than one male and thus, reduce the strength of sexual selection operating on males.

Several hypotheses in addition to sexual selection propose adaptive mechanisms to explain the variation in SSD in animal species. Differential costs to the sexes (such as energy allocation and susceptibility to predation) as a result of their reproductive activities can contribute to the observed degree and direction of SSD (Carothers 1984). Reduction of intersexual competition through differential use of resources between the sexes (Schoener 1966, Selander 1972, Shine 1989a, 1991) as well as variation in ecological and environmental factors between geographically separated populations (Iverson 1985, Shine 1989a) have also been proposed as potential influences on SSD between and within species. Another determinant of size differences between the sexes may be due to the 'fecundity advantage' (Darwin 1874) or 'big mother' hypothesis (Ralls 1976). Larger females have more offspring in a variety of taxa (invertebrates: Wiklund and Karlsson 1988; anurans: Woolbright 1983; reptiles: Berry and Shine 1980, Fitch 1981; birds: Hughes and Hughes 1986) selecting for large female body size. Thus, females may be larger than the males of a species as a result of natural selection. In Chapter Four, I examine the influence of mortality, energetic constraints and fecundity advantage on the observed degree and direction of SSD in northern water snakes.

For practicality, I examine each hypothesis (non-adaptive, sexual selection and natural selection) separately. In reality, the relative sizes of males and females are probably determined by the action of multiple factors simultaneously (Ralls 1976, O'Neill 1985,

Arak 1988, Hedrick and Temeles 1989). In the final discussion, I incorporate all potential contributing factors that I have examined to propose an explanation for the observed degree and direction of size dimorphism in northern water snakes.

Study Organism

Northern water snakes (family: Colubridae) are a common species within their range which extends from Maine to Colorado and from Tennessee to southern Ontario and extreme southwestern Quebec (Conant 1975). Water snakes commonly inhabit swamps, marshes and bogs, streams, ponds and lakes. Although this semi-aquatic species is highly dimorphic in size (females being the larger sex), there is no difference in colour between sexes. They both have a dark brown or greyish dorsum with the ventral surface being light and highly variable in the patterns and colours.

In its northern range, the northern water snake is active between mid-April upon emergence from hibernation to October when cold weather restricts their movements (King 1986). The breeding season extends from late April to early June, depending on temperature. Females give birth to live young in late August and into September (Conant 1975).

CHAPTER ONE

The Effect of Allometry and Phylogeny on Sexual Size Dimorphism in Snakes

Introduction

Studies investigating differences in SSD or in life history traits among species have generally focussed on the adaptive significance of the traits. Recently, however, the importance of potentially non-adaptive forces on the evolution of life history traits has been recognized (Brown 1983, Cheverud et al. 1985, Fairbairn 1990, Shine 1991). The observed patterns of interspecific variation in life history traits or in SSD may be a result of both adaptive and non-adaptive forces. Shine (1981) suggested that in order to determine how adaptation to the immediate environment explains the variation in life history traits, it is first necessary to factor out the effects of other, potentially non-adaptive, processes. In this chapter, I examine the potential influence of allometry and phylogeny on the patterns of SSD in snake species.

Many characteristics of an animal species may be constrained by allometry, i.e., the change in proportion of various parts of an organism as a consequence of a change of body size. Allometric relationships have been well documented in a wide diversity of taxa (e.g. Peters 1983). A general trend of increasing sexual dimorphism with body size is exhibited in a wide diversity of species that exhibit male-biased size dimorphism (e.g. primates, Clutton-Brock et al. 1977, Cheverud et al. 1985; terrestrial turtles, Berry and Shine 1980; grouse, Wiley 1974 and fig wasps, Hamilton 1979). For

species in which males are the smaller sex, the difference in body size between the sexes decreases with increasing body size (Wheeler and Greenwood 1983). Thus, in general, for a given increase in species size, males increase in size relative to females (Fairbairn 1990).

Both Stearns (1984) and Dunham and Miles (1985) found that species size (indexed by female body length) explains a significant proportion of the variation in several life history traits in reptiles. Since female-biased size dimorphism predominates in snake species (Fitch 1981), one would expect that if snakes follow the general allometric pattern, males of smaller snake species would be relatively smaller than females than would males of larger species. My first objective is to examine the extent to which snakes follow this general trend.

Phylogeny may also influence patterns of sexual size dimorphism if adaptational changes in body size of a species are constrained by its evolutionary history (Stearns 1984, Cheverud et al. 1985). A particular snake species may have a higher degree of size dimorphism than others simply because its ancestor was more dimorphic than average. This is a particularly important consideration in interspecific comparisons. Phylogeny accounts for a high proportion of the observed interspecific variation in body size in many taxa (e.g. snails, Brown 1983; primates, Cheverud et al. 1985; mammals, Stearns 1983). Thus, the presence or absence of this 'phylogenetic inertia' (Dunham and Miles 1985) should be assessed before other hypotheses explaining size dimorphism are

investigated. My second objective is to examine the effect of phylogeny on patterns of sexual size dimorphism observed in snakes.

Methods

From the literature, I obtained values of average male and female body size for 82 snake species in four families. As the estimate of SSD of a species, I used the ratio of female snout-vent length (SVL) to male SVL multiplied by 100. A female to male ratio (FMR) value of 100 for a species indicates that males and females are of equal size. Values greater than or less than 100 indicate a difference in body size between the sexes. All estimates of FMR values were taken from Fitch (1981) with the exception of *Thamnophis butleri* (Ford and Killebrew 1983) and *Bothrops asper* (Solorzana and Cerdas 1989). I included only those species for which measurements of average male and female SVL were based on data from more than 10 individuals. Using one main source should have minimized confounding errors due to inter-observer measurement bias. I used SVL as a measurement of body size since tail damage, common in nature, renders total length measurements inaccurate. As well, tails are relatively longer in male reptiles than in females which would confound the size difference (Fitch 1981).

The taxonomic relationships between the four families - Boidae, Colubridae, Elapidae and Viperidae - are shown in Figure 1-1. Several species of Elapidae have previously been classified in the family Hydrophiidae (Fitch 1981, Seigel and Fitch 1984). However, recent systematics have placed these species as a subfamily of Elapidae (McDowell 1987). The Crotalidae, treated as a separate

Figure 1-1 Phylogenetic relationships between four families of snakes (from McDowell 1987).

family by Fitch (1981), have been placed as a subfamily of Viperidae following McDowell (1987). Colubridae and Viperidae are more closely related to each other than either is to Elapidae, and all three are more closely related to one another than any are to Boidae.

To determine if snakes follow the general allometric pattern exhibited by other taxa (i.e. males increase in size relative to females with an increase in body size), I investigated the extent to which body size influences sexual size dimorphism in snakes. I performed a regression of FMR on species size using the female SVL as the index of species size. Next, I tested for the presence of phylogenetic constraints by assessing the effect of family on FMR while controlling for species body size (analysis of covariance - ANCOVA). Since the sample size of the family Boidae was small (N=2), inter-family comparisons were restricted to the remaining three families.

Results

Sexual size differences among the 82 species ranged from highly male-biased (FMR = 77) to highly female-biased (FMR = 146). This range is slightly larger than ranges reported for some other animal groups (e.g. primates 98-146, Alexander et al. 1979; waterstriders 98-118, Fairbairn 1990), indicating a relatively high variance in SSD within snakes. Female-biased dimorphism (i.e. FMR values over 100) is most prevalent in the families Colubridae and Elapidae while in Viperidae species, males are generally the larger sex (Table 1-1).

Table 1-1 Distribution of sexual size dimorphism (FMR - female SVL to male SVL ratio) for species within four snake families

Family	N	% of species			FMR $\bar{X} \pm SD$
		M > F (FMR < 98)	M = F (98 > FMR < 102)	M < F (FMR > 102)	
Boidae	2	-	-	100	125.5±16.3
Colubridae	33	27	12	61	107.3±13.2
Viperidae	31	71	10	19	94.8±8.9
Elapidae	16	25	25	50	110±17.2

Allometry and Phylogenetic Constraints

There was a significant negative correlation between FMR and species size (as indexed by female SVL) ($r^2=.097$, $F=6.58$, $df=80,1$, $p=.01$) (Figure 1-2). This supports the general trend that in larger species, males are larger relative to females than are males in smaller species. However, there is a great range in FMR values at any given body size (Figure 1-2). Indeed, only 9.7% of the variance in FMR is explained by allometry.

I next performed an analysis of covariance of mean SSD values by family while controlling for female body size. The interaction between mean female SVL and family is not significant. However, there is a significant difference in FMR values between families (ANCOVA $F=10.31$, $df=76,2$, $p<.001$) (Figure 1-3). Difference due to families accounts for 19% of the total variation in FMR across species (sums of squares of families/ sums of squares of total).

Discussion

Snakes, in general, exemplify the allometric trend that, for a given increase in species body size, males have a relatively greater increase in size than females. As well, the degree and direction of SSD observed in a species can be partially explained by phylogeny. While this difference between families may be a result of phylogenetic constraints, I cannot exclude the possibility that this difference reflects an adaptational response to common environmental conditions within families.

There is wide variation in SSD values among snake species. Allometry and the difference between families accounts for only a

Figure 1-2 Sexual size dimorphism (FMR- female SVL to male SVL ratio) relative to species size (indexed by mean female SVL) by family. Regression line indicates simple fit of total sample (N=82). + represent Colubridae (N=33), × Viperidae (N=16), o Elapidae (N=31), ● Boidae N=2).

Figure 1-3 Differences between families in the relationship between SSD and species size. Regression lines of simple fit for Viperidae represented by ---; for Elapidae ---; and for Colubridae Solid line represents line of simple fit for all families combined.

small proportion of this variation. Thus, although the influence of these two non-adaptive forces of size dimorphism in snake should be taken into account, the majority of the variation in sexual size dimorphism observed in snake species (at least 70%) remains unexplained. Some of this unexplained variation may be a result of adaption. In the next three chapters, I will be examining several adaptive explanations for the pattern of SSD in one snake species, the northern water snake.

CHAPTER TWO

Reproductive Biology of the Northern Water Snake

Introduction

Many studies have advocated the role of sexual selection in determining patterns of SSD among species (e.g. Shine 1978, Wade 1979, Price 1984). Sexual selection refers to the selection for any characteristic, such as large body size, that enhances an individual's ability to secure mates and thus, increase its reproductive success (Darwin 1871, Trivers 1972). Operation of sexual selection is governed by the relative investment of the sexes in their offspring (Trivers 1972). In the majority of mammalian and avian species, the male invests relatively little (compared to the female) in his offspring past the fertilization of the egg. Since females are the limiting factor to males attaining high reproductive success, males usually compete for females. Alternatively, although quite rarely, males invest more in the offspring than do females (in the form of parental care) and females compete amongst themselves for males (i.e. spotted sandpiper, *Actitis macularia*, Oring 1981). In this chapter, I test hypotheses regarding sexual selection and the pattern of SSD in northern water snakes.

In many species, such as snakes, in which the male's only contribution to his offspring is sperm, males compete intensely to obtain matings with females. This strong competition would result in strong sexual selection operating on males. If large size is advantageous to gaining and maintaining a resource attractive to females or to establishing high dominance status, then large males

would mate more often, thereby passing along genes for large body size. Assuming that females are at the optimal body size for the species, sexual selection for large male body size is the chief explanation for the presence of male-biased size dimorphism in many species (Shine 1978, 1979b, Clutton-Brock et al. 1977, Price 1984, LeBoeuf and Reiter 1988). Thus, one explanation for the female-biased size dimorphism reported for northern water snakes may be that males are not experiencing strong sexual selection and that other selection pressures (i.e. natural selection for large females or for small males) result in females being the larger sex. My first objective was to investigate the hypothesis that male northern water snakes do not experience strong sexual selection.

The strength of sexual selection acting on males is a function of the intensity of the competition among males for mating opportunities (Wade 1979). Male-male competition is intense when only a few males acquire access to females. This can occur if there are fewer receptive females in the population than there are males available to mate (i.e. that the sex ratio is male-biased). It has been reported that water snakes form mating aggregations in which many males appear to court a single female simultaneously (Tinkle and Liner 1955, Mushinsky 1978). If these aggregations are rare and the ratio of sexually active males to females is usually equal, then competition between males may not be intense. Thus, there may not be strong sexual selection pressures on males. To test the hypothesis that males are not experiencing strong sexual selection, I tested the prediction that male-male competition in northern water snakes is not pronounced by examining sex ratios of various

segments of the population. I first determined the sex ratio of the study population as a whole. I then examined the breeding sex ratio, i.e. the sex ratio of all sexually mature individuals in the population, in order to assess the potential competition between males for opportunities to mate. To get the most accurate view of the intensity of male-male competition, I also determined the sex ratio of all individuals that were actively mating in the population at any given time. This last sex ratio is called the operational sex ratio (OSR) (Emlen and Oring 1977). As I captured snakes to determine their sex, I obtained measurements of body length and weight. Thus, I also assessed the extent of SSD to confirm that this population of water snakes is size dimorphic.

Another explanation of female-biased size dimorphism may be that sexual selection is acting on males but that it is not large body size that enhances success in intra-sexual competition. Males should only grow as large as females if male reproductive success increases with body size (Shine 1979b). Large male size is favoured in some animal species because males can force copulations (Ralls 1977, Berry and Shine 1980, Alcock 1984). However, such an advantage to large body size is unlikely to apply to snakes. Body shape and lack of appendages would limit the ability of a male snake to restrain a female to force copulations (Devine 1984). Alternatively, other traits, such as long tails or good body condition, may be more advantageous than large size to a male to obtain matings. For example, it has been suggested, but not substantiated, that increased male tail length increases copulatory effectiveness in snakes (Semlitsch and Gibbons 1982). As well, males in good

condition may be better competitors. For instance, they may be able to participate in more mating aggregations than males in poor condition because they could spend less time foraging. In attempting to explain the female-biased size dimorphism in northern water snakes, my second objective was to test the hypothesis that sexual selection favours some male trait(s) other than body size.

Methods

I studied a population of northern water snakes within 10 km of the Queen's University Biological Station near Chaffey's Lock in eastern Ontario. The study area consisted of mixed forests, fields and wetlands. A small beaver marsh (approximately 0.5 km²) (hereafter referred to as Barb's marsh) within the study area was chosen as the site for detailed field observations. This marsh had the advantage of being the site of water snake studies in the two years previous to my study. Thus, many of the snakes had been caught and marked. The habitat in Barb's marsh consisted of a mixture of open water, willows (*Salix sp.*) (dead trees and stumps), two beaver lodges in the middle and grasses and cattails around the edges.

In both years of my study, I attempted to catch and mark as many snakes as possible before the breeding season started. Therefore, between 22 and 30 April 1990 and 25 and 30 April 1991, I visited the marsh at least once daily and walked randomly through the marsh attempting to capture all snakes seen. All captured snakes were weighed (to the nearest g) and the total and vent to tail

tip lengths were measured (to the nearest 0.1 cm) to calculate snout-vent length (SVL). Male snakes have relatively longer tails than females (King 1989) which would confound comparisons of total length between the two sexes. I calculated the condition of each individual as $\text{weight} / (\text{SVL})^3$. Sex was determined by probing the cloaca for the presence of hemipenes. Each individual was branded in a unique pattern on the scales posterior to the vent to provide a permanent mark. In addition, I gave the snakes temporary marks along their backs using spots of acrylic paints in unique colour combinations. These paints remained identifiable throughout the breeding season and enabled me to recognize individuals without having to disturb them on each sighting.

I defined the breeding season as the time between the first and last day that I observed mating aggregations. In 1990, the breeding season lasted from 30 April - 3 June. In 1991, the first and last mating aggregations were observed on 30 April and 24 May, respectively. During the breeding season in 1990, parts of the marsh were searched daily and the whole marsh an average of three times a week by walking transects approximately 3 m apart and recording sightings of snakes. In 1991, I walked transects, again approximately 3 m apart, of the whole marsh at least once a day. If multiple sightings of an individual occurred during a search, only the first observation was included in any analysis. On every visit to the marsh in both years, I recorded temperatures of the air (in the shade) and water (approximately 10 cm depth). I used a hand held thermistor thermometer (Cole Parmer Instrument Co.) with a silicon tipped probe.

It was important to distinguish mating aggregations from observations where snakes were aggregating but not mating. This proved to be unambiguous since water snake mating behaviour is very distinctive. A male water snake attempting to mate with a female moves along her back and wraps his tail around hers until their cloacas are aligned. Any other males involved in the mating aggregation constantly move among the entwined group of snakes. Another distinctive feature is that during mating activity, the males are observed twitching their heads up and down and rapidly flicking their tongues. Periodically, the males also give spasmodic jerks which are forceful enough to move the female and any other males entwined in the mating aggregation. The female is passive during courtship and mating and can terminate mating activity at any time simply by leaving the area. These observations are consistent with those of mating snakes reported in the literature (Hammerson 1978, Mushinsky 1978, Perry-Richardson et al. 1990). I recorded all mating aggregations I encountered, noting the number and sex of all participants. I defined an aggregation as a mating aggregation when: 1) two or more snakes were intertwined and I could see two tails wrapped together with the cloacal areas against each other, 2) at least one male was head twitching or 3) the pile of snakes periodically jerked. Since I observed a high density of snakes (both mating and not mating) on the beaver lodges, I noted the location of the mating aggregations as occurring either on the lodges or elsewhere in the marsh. Since I was unable to observe actual intromission, I used the number of mating aggregations in which a male participated as an estimate of potential male mating success.

To ensure that my sample of the population was not biased by the mating season, I continued to walk transects through the whole marsh and attempted to capture all unmarked snakes at least four times a week from after the breeding season ended until 7 July 1990 and 26 June 1991. I determined the population sex ratio from all captures of water snakes before, during and after the breeding season.

I defined the breeding sex ratio as the ratio of all sexually mature males and females in the population. Since I assumed that all snakes seen in mating aggregations were sexually mature, I defined the size of sexual maturity for both sexes as being the SVL of the smallest individual of each sex observed in any mating aggregation. I tested for sperm presence in the majority of males captured in both years (68% of total males captured in 1990 and 96% in 1991) as an additional method for determining size at sexual maturity in males. Following a method described by Charland (1987), I palpated the abdomens of the males towards the vent several times. A cotton swab, soaked in saline solution, was gently inserted into the cloaca and then smeared onto a microscope slide. The procedure called for staining in order to see the sperm. However, I found that sperm was easily visible at 100x magnification without the stain. Thus, that step was eliminated from my protocol.

The operational sex ratio (OSR) is the ratio of males and females actively mating in the population at any given time (Emlen and Oring 1977). I determined the OSR of northern water snakes in Barb's marsh by recording the total number of individuals of both

sexes involved in all aggregations on any given visit to the marsh and then averaging this over the breeding season.

To assess whether the population in Barb's marsh was representative of water snakes in the general study area, I obtained data on water snakes from two additional sources for comparison. I captured water snakes in a marsh (called Beaver marsh) approximately 2 km from Barb's marsh. I searched this marsh at least three times a week during May 1990 and at least once a week during May 1991. All snakes captured were measured and marked with a permanent mark similarly to those in Barb's marsh. The only exception in the protocol was that I did not apply the acrylic paint markings to these individuals. The second source of comparative data came from the records of the National Museum of Canada, Herpetology section in Ottawa, Ontario. I gathered data on the lengths and sex of specimens of water snakes collected in southeastern Ontario and western Quebec, the region of my study site.

I used a microcomputer version of the Statistical Package for the Social Sciences (Nie 1988). For each analysis, I tested that the data were normally distributed and used non-parametric statistics when they were not. All means are reported $\pm$ one standard deviation.

Results

Over the two years of this study, 117 northern water snakes were captured in Barb's marsh. Eighty-nine snakes (51 males and 38 females) were captured in the 1990 field season and 71 snakes (43

males and 28 females) were captured in 1991. Forty-three (24 males and 19 females) of these individuals were captured in both years. In both years, the majority of snakes had been marked by the end of the breeding season. During this period, I saw approximately one unmarked snake per 50 observed. Thus, the total captures included most of the population of northern water snakes in Barb's marsh.

The paint markings were bright enough to allow me to identify individuals from a distance. This leads to the question of whether the paint increased the susceptibility of the snakes to predation. Of the 71 snakes caught in 1991, 43 were recaptured from the previous year when the snakes had first been painted. This recapture rate is comparable to the year before when, of the 89 snakes caught in 1990, 50 were recaptures from 1989 when snakes were not painted ($X^2 = .08$, $df = 1$, $p = .77$). Thus, snakes with paint were as likely to be recaptured as snakes without paint, indicating that the paint markings do not appear to increase susceptibility to predation.

Sexual Size Dimorphism

Female water snakes were significantly 'bigger', on average, than males for total length, snout-vent length (SVL) and weight within Barb's marsh (Table 2-1). Both years of the study were analyzed separately to examine differences between years and also because some individuals were caught in both years. Comparison between males and females captured in Beaver marsh and data collected from museum records (Table 2-1) similarly showed that females are 'bigger', on average, than males. This similarity

Table 2-1 Comparison (two-tailed t-test) of the morphology between male and female northern water snakes from three sources. Values shown are mean±(SD).

	N		Total length (cm)		Snout-vent ler.gth (cm)		Weight (g)	
	M	F	M	F	M	F	M	F
Barbs								
1990	51	38	68.3 (9.5)	75.6 * (17)	51.3 (7.2)	59.2 ** (13)	100.2 (36.5)	153.5 ** (81.7)
1991	43	28	69.9 (9.7)	81.4 ** (16.6)	52.4 (7.8)	63.2 ** (12.8)	103.5 (36.1)	207.2 ** (102)
Beaver								
1990	49	41	68.5 (10.7)	88.0 ** (17.0)	51.4 (8.1)	68.7 ** (13.7)	105.3 (41.4)	273.5 ** (136.1)
1991	12	10	74.9 (6.8)	86.9 (20.8)	55.7 (5.1)	69.1 (16.7)	112.3 (36.3)	284.3 ** (141.4)
Museum	51	38	69.3 (18.7)	89.5 ** (27.7)	52.5 (14.6)	71.6 ** (22.6)	/	/

Levels of significance * $p < 0.05$, ** $p < 0.001$

between samples from different sources indicates that the population in Barb's marsh is representative of water snakes in the region.

Although females attain larger sizes than males, at a given body size (SVL), weights of males are within the ranges of female body weight (Figure 2-1) (1990 Male $Y = .002x^{2.71}$, $r^2 = .89$, Female $Y = .002x^{2.69}$, $r^2 = .95$; 1991 Male $Y = .006x^{2.44}$, $r^2 = .92$, Female $y = .0004x^{3.09}$, $r^2 = .94$). It appears that the difference in average body size and average weight between the sexes is a consequence of male water snakes rarely exceeding a SVL of 70 cm. Females grow longer (SVL greater than 80 cm) and, since weight increases approximately as the cube of SVL (Figure 2-1), females become substantially larger than males in volume and weight.

It is possible that the size bias is just a consequence of higher male mortality creating a skewed age structure (i.e. more older females). Feaver (1977) created an age/size curve for *Nerodia sipedon* based on the integration of the growth rates of 34 females and 21 males. Using this length-age curve, I constructed an approximate age distribution for snakes in Barb's marsh (Figure 2-2). Males have a similar age distribution to females (Kolmogorov-Smirnov two sample test 1990: $Z = .778$, $p = .58$; 1991: $Z = 1.02$, $p = .25$). Thus, the rarity of large males is not simply a consequence of males failing to live long enough to grow large. It appears that the size bias in the population is real and not due to an age bias.

An independent approach to confirm that the size bias of water snakes is not a consequence of males suffering higher mortality than females is to examine the recapture data from previous years in

Figure 2-1 Comparison between males (+) and females (o) of the relationship between body size (measured as snout-vent length) and weight. for (a) 1991 (N=43 males N=28 females) and (b) 1990 (N=51 males N=38 females) .

Figure 2-2 Age distribution of northern water snakes in Barb's marsh in (a) 1990 and (b) 1991. Ages estimated from age/size curves determined by Feaver (1977). Striped bars represent males, solid bars females.

Barb's marsh. The population of water snakes in Barb's marsh had been studied in 1988 and 1989, the two years previous to my study. Thus, there were four years of data from which to compare recapture rates of males and females. Snakes recaptured two years after the year of first capture included individuals first caught in 1988 (recaptured in 1990) and those first caught in 1989 (recaptured in 1991). Approximately, 11% (11/98) of all males and 7.2% (4/55) of all females caught in both 1988 and 1989 were recaptured two years after the first year of capture. Out of the 57 males caught in 1988, a further 11 (19%) (not including those recaptured only two years after year of first capture) were recaptured three years later in 1991. Only three females of the original 27 (11%) caught in 1988 were recaptured in 1991. There is no significant difference between the sexes in recaptures two or three years after the year of first capture ($X^2 = .28$, $df=1$, $p=.6$; $X^2=.47$, $df=1$, $p=.49$ respectively) . Thus, females are not more likely than males to be recaptured in later years, contrary to expectations if males suffered higher mortality. Thus, this population of northern water snakes is genuinely sexually size dimorphic in that males are significantly smaller than females at the same age.

Sexual Selection

Sexual maturity

Of those females captured during the breeding season, no female under a SVL of 58.7 cm was observed in a mating aggregation in either year of study (Figure 2-3a). Thus, I defined females at or greater than this length as being sexually mature. Two estimates of

Figure 2-3 Number of (a) females and (b) males in each size class observed during the breeding season of 1990 and 1991 combined either mating or not mating. Striped bars represent number of snakes not observed mating; solid bars number of snakes observed in mating aggregations.

size at maturity from other populations of water snakes were available from the literature. King (1986) and Feaver (1977) determined that, while some smaller females were sexually mature, all females with a SVL larger than 59 and 65 cm, respectively, are sexually mature. My estimate of female size at sexual maturity based on observation in mating aggregations is comparable.

Of all males captured during the breeding season, the smallest male that was observed in a mating aggregation had a SVL of 42.5 cm (Figure 2-3b). The smallest male testing positive for sperm was 37.5 cm (SVL). This male was also the smallest male that was tested for the presence of sperm. Thus, the minimum size of maturity defined by the sperm sampling is not conclusive since males smaller than 37.5 cm SVL could have had sperm. Approximately 25 and 9% (1990 and 1991 respectively) of the males larger than 37.5 cm SVL tested negative for sperm presence. As most of these males were either observed in mating aggregations or had sperm in 1991, this technique appear to underestimate the presence of sperm. However, sperm sampling did corroborate that the smallest male observed in a mating aggregation was sexually mature.

My estimate of size of 42.5 cm SVL for sexual maturity for males based on participation in mating aggregations is close to other estimates reported in the literature. King (1986) and Feaver (1977) determined that, while some smaller males were sexually mature, all males with a SVL larger than 43 and 42.5 cm, respectively, are sexually mature. In all subsequent analyses, I will

only include sexually mature individuals of both sexes unless otherwise stated.

Sex ratios

The sex ratio of the population in Barb's marsh for both years was male-biased, although not significantly (Table 2-2). When I combined the years (noting that the two years are not truly independent), the population sex ratio is significantly male-biased. This result is similar to that found by King (1986) for the Lake Erie water snake (*Nerodia sipedon insularum*) (M:F 1.13:1). As part of another study done in 1990 (Chapter three), I obtained broods of water snakes from 14 females. The natal sex ratio was not significantly different from unity ($N=320$, M:F 1:1.15, $X^2 = 1.51$, $p=.219$). Thus, there appeared to be fewer females than males in the population even though the sex ratio at birth was equal. For both years, I compared the population sex ratios at successive age intervals for all the snakes captured in the year. If females are subject to higher mortality, then a population trend of increasing male bias with an increase in age would be expected. Although not significantly different from zero, the slope of the regression for both years combined was positive (Figure 2-4). Male bias in the population sex ratio did increase slightly with age.

The breeding sex ratio (the ratio of sexually mature males and females) was slightly more male-biased than the population sex ratio, albeit not significantly (Table 2-2). This may be explained by the fact that male water snakes mature earlier than do females. From the age/size curve mentioned previously, I estimated that

Table 2-2 Population, breeding and operational sex ratios (male: female) of northern water snakes in Barb's marsh.

Sex Ratios	1990		1991		Combined	
	N	MF	N	MF	N	MF
Population	89	1.34:1 NS	71	1.59:1 NS	160	1.4:1 *
Breeding	75	1.77:1 * Pop NS	60	2.0:1 ** Pop. NS	135	1.9:1** Pop NS
Operational	176	2.74:1 ** BSR * Pop **	70	2.3:1** BSR NS Pop NS	246	2.6:1 ** BSR * Pop **

Chi-square test of observed sex ratio against

1) sex ratio of 1:1

2) breeding sex ratio (BSR)

3) population sex ratio (Pop)

Levels of significance are * $p < 0.05$, ** $p < 0.001$

NS - non-significant

Figure 2-4 Change in sex ratio (males:female) with age for both 1990 (+) and 1991 (■) combined. Numbers above each data point are the total number of individuals at each age.

males in Barb's marsh mature at 24 months, much earlier than do females at 36 months. This may help to explain the male-biased breeding sex ratio. The total number of sexually mature males includes individuals from the second year cohort while the total number of mature females does not.

Operational sex ratio

The operational sex ratio (the ratio of males and females participating in mating aggregations at any given time) for both years separately was significantly male-biased (Table 2-2). I observed mating aggregations of water snakes throughout the marsh in a variety of habitats - branches of willow trees, on stumps, on grass clumps and on the two beaver lodges. The highest density of snakes occurred on the beaver lodges (both mating and non-mating individuals). The average sex ratio of all mating aggregations observed was male-biased (Table 2-3). Although several matings involved just one male and one female, the sex bias in one aggregation was as high as 12 males to one female. In 1990, most of the mating aggregations occurred on the beaver lodges where the operational sex ratio was highly male-biased (3:1 M:F). In 1991, mating aggregations occurred more equally throughout the marsh but there were still more males than females actively mating (OSR 2.3:1).

Despite the difference between years of where the aggregations occurred in the marsh, the overall operational sex ratio was significantly male-biased both years. Thus, although there were equal numbers of males and females at birth, the number of

Table 2-3 Operational Sex Ratio (male:female) by location of mating aggregations in Barb's marsh. Locations are on beaver lodges or elsewhere in the marsh.

Operational Sex Ratio	1990		1991		Combined	
	N	MF	N	MF	N	MF
Lodges	153	3.1:1 **	25	2.1:1NS	178	2.9:1**
Elsewhere	23	1.3:1NS	45	2.5:1NS	68	1.9:1NS
Overall	176	2.74:1**	70	2.3:1**	246	2.6:1**

Chi-square tests of significance are against a sex ratio of 1:1

Levels of significance are * $p < 0.05$, ** $p < 0.001$

NS - non significant

actively mating males outnumbered the females by almost 2:1. Since the breeding and population sex ratios were male-biased, part of the difference in numbers of actively mating males and females can be explained by the fact that there are more sexually mature males than females in the population. However, this is not the full explanation because the operational sex ratio is significantly more male-biased than both the breeding and population sex ratios (Table 2-2).

A possible explanation for the highly male-biased OSR is that female water snakes may breed only every second year (biennial reproduction). Thus, on average, in a given breeding season only half of the sexually mature females may be receptive. In 1990, I observed 17 of the 25 mature females in mating aggregations. In 1991, eight of 20 mature females were observed in mating aggregations. These results are probably underestimates since I almost certainly did not observe all mating aggregations. Additional data from the captive females (Chapter Three) showed that 14 of the 20 (70%) females in captivity gave birth. Thus, over half of the females in the population do reproduce in a given year indicating that annual reproduction is common. However, it appears that some females may not reproduce every year, contributing to the male-biased OSR.

Another explanation of the highly male-biased OSR may be that females do not mate as synchronously as males. Thus, at a given time in the breeding season, only a few mature females may be actively mating while many mature males are attempting to mate, creating a highly male-biased operational sex ratio. I examined two

components of sexual activity to determine whether females mated more asynchronously than males. First, I compared the date of first mating within the sexes. Although neither sex started mating completely synchronously (Figures 2-5 and 2-6), males appeared to start slightly more synchronously than females. Thus, differences in the onset of mating activity within each sex may contribute slightly to the male-biased OSR.

I also compared the duration of sexual activity (the time between the first and last day I observed an individual in mating aggregations) between sexes. Both males and females varied in the duration of sexual activity (1-23 days and 1-6 days, respectively). In 1990, males were active for an average of 12.5 ± 10.1 days ($N=31$) while females were observed mating for an average of 5.5 ± 6.5 days ($N=18$). Thus, not only were there more males mating but these males were active for much longer than females during the breeding season (Mann-Whitney U-test, $Z=2.5$, $p=.011$). This would increase the male-bias in the OSR. In 1991, males and females were sexually active for similar lengths of time (4.3 ± 4.9 days ($N=19$), 2.75 ± 3.8 days ($N=8$), respectively: Mann-Whitney U-test, $Z=.16$, $p=.87$). Thus, males in 1990 were sexually active for more days and for a longer time relative to the females than were the males in 1991. This may partly explain why the OSR is much higher in 1990 than in 1991.

Since only one female participated in each mating aggregation, the number of females mating each day is the same as the number of mating aggregations occurring. The number of mating aggregations per day varied throughout the breeding season (Figure 2-5). A possible explanation of the different number of mating

Figure 2-5 Estimates of duration of sexual activity for females in Barb's marsh in 1990 and 1991. Dots indicate days on which females were observed in mating aggregations.

Figure 2-6 Estimates of duration of sexual activity for males in Barb's marsh in (a) 1990 and (b) 1991. Dots indicate days on which males were observed in mating aggregations.

a)

Male

1990

Date (May - June)

30 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 1 2 3

b)

Male

Date (May)

4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

1991

1 0-----0--0

2 0-----0

3 0-----0

4 0--0-0-----0-----0

5 0-----0

6 0

7 0--0-0

8 0--0-0

9 0--0-0

10 0--0-0

11 0

12 0-----0

13 0

14 0

15 0

16 0-----0

17 0

18 0

19 0--0

aggregations occurring each day may be that weather influences the mating behaviour of northern water snakes. As an example from Figure 2-5, a high number of mating aggregations was observed on the 14th of May 1990. The weather on that date may have provided optimal conditions for water snake mating. However, I found no significant relationship between air or water temperature and the number of females mating through the breeding season in either year (range 0-6; for 1990: ANOVA $F=1.35$, $df=18,3$, $p=.28$; for 1991: $F=.767$, $df=17,3$, $p=.52$) or the number of males participating in mating aggregations on a particular day (range 0-18; for 1990: ANOVA $F=1.36$, $df=18,3$, $p=.28$; for 1991: $F=.43$, $df=17,3$, $p=.73$). Thus, I could not explain within-year variation in mating aggregations by the environmental factors I measured.

Weather differences between the years could possibly explain why males were sexually active longer in 1990 than in 1991. However, I was unable to find any significant difference between the years in mean air and water temperature or in mean cloud cover (Table 2-4). Differences may lie in factors that I did not measure, such as wind speed or overnight air temperature. However, even with the variation in the start and duration of mating activity of both sexes both between and within years, the overall OSR was significantly male-biased.

Correlates of Male Mating Behaviour

One strategy for a male to increase his reproductive success would be to mate alone with a female and avoid the competition of other males in larger mating aggregations. To investigate if males

Table 2-4. Comparison of air and water temperatures (° C) and cloud cover (tenths) between years in Barb's marsh. Values shown are means $\pm$ SD.

	1990	1991	F statistic ^a	P value
Air Temperature	17.19 $\pm$ 4.8	19.16 $\pm$ 18.7	.40	.531
N	24	22		
Water Temperature	16.44 $\pm$ 3.5	17.56 $\pm$ 3.21	2.09	.156
N	25	22		
Cloud Cover	5.35 $\pm$ 3.8	3.47 $\pm$ 3.8	3.11	.085
N	23	21		

^a analysis of variance (ANOVA)

mating alone with a female have certain traits that enhance their reproductive success. I performed an analysis of variance using several male morphological traits (relative tail length, SVL and condition) and compared males that were observed mating alone with a female with those that were always observed in mating aggregations with other males. Males that were observed mating alone with a female were not significantly different from males only found in mating aggregations with more than one male (Table 2-5).

Another way that a male could increase his reproductive success, would be to increase the number of mating aggregations in which he participates. To determine if the difference between males in the number of opportunities to mate is correlated with any morphological trait, I performed a multiple regression on the number of mating aggregations in which a male was observed using relative tail length (tail length/total length), SVL and condition as independent variables for all sexually mature males captured before the end of the breeding season. In 1990, larger males and males in better condition were observed in more aggregations (df = 38; relative tail length: $t=1.19$, $p=.24$; SVL: $t=3.17$, $p=.003$; condition: $t=3.45$, $p=.001$). In 1991, there was no significant relationship between any trait and the number of aggregations in which a male was observed (df = 31; relative tail length: $t=1.09$, $p=.28$; SVL $t=1.15$, $p=.25$; condition $t=-1.36$, $p=.18$). For both years combined, both condition and SVL were significantly correlated with the number of aggregations in which a male was observed (df=77, relative tail length $t=.99$, $p=.31$, SVL $t=2.59$, $p=.011$, condition

Table 2-5 Comparison between males mating alone with a female and males involved in mating aggregations (MA) with several males. Values are means $\pm$ one SD.

	Males alone with female	Males in larger MA	F statistic ^a	p value
1990				
N	6	9		
RTL	.26 $\pm$.01	.25 $\pm$.01	3.64	.079
Condition	7.46 $\pm$.9	7.2 $\pm$ 1.16	.211	.654
SVL	48.0 $\pm$ 3.7	52.1 $\pm$ 5.1	2.85	.115
1991				
N	10	8		
RTL	.24 $\pm$.05	.25 $\pm$.013	.968	.34
Condition	6.82 $\pm$ 1.25	7.01 $\pm$ 1.03	.116	.737
SVL	55.62 $\pm$ 9.4	54.41 $\pm$ 7.2	.09	.768
Combined				
N	16	17		
RTL	.25 $\pm$.01	.25 $\pm$.01	.437	.513
Condition	7.06	7.11	.016	.899
SVL	52.76	53.19	.028	.869

^a Analysis of variance (ANOVA)

* RTL - relative tail length = tail length / total length

Condition = weight / (SVL)³

SVL - snout-vent length (cm)

$t=2.33$, $p=.02$). Thus, larger males and males in the best condition would have more chance for success simply by participating in more aggregations.

It appears that large males in the best condition had the greatest potential for high reproductive success. The importance of condition may explain why males are limited to a body size smaller than that of females even though larger males are more successful. There may be an optimal body size that allows males to maintain the best condition given the availability of the food resources. Condition during the breeding season will be determined by the extent of feeding a male does before and during the breeding season. Larger males are in more mating aggregations. However, a longer individual would need a greater weight gain and therefore more food, to maintain the same condition as a smaller individual. Thus, there may be an optimum body size for males below which they are not large enough to mate effectively no matter what their condition, and above which it would be energetically costly (in terms of time of foraging required) to maintain a high body condition.

Although I cannot use my data to test the hypothesis that males have an optimal size determined by condition (since these data are not independent of those used to generate the hypothesis), I can examine my data to see if they are consistent with this hypothesis. The hypothesis predicts that if there is an optimal body size beyond which it is costly to maintain good condition, then large males should be in poorer condition. To test this prediction, I examined the relationship between condition and SVL for all males captured in 1990 and 1991 (Figure 2-7). In both years, condition

Figure 2-7 Relationship between length (SVL) and condition of male northern water snakes in 1990 (■) (N=45) and 1991 (o) (N=36). Regression lines for 1990 represented by dashed line and for 1991 by solid line.

declined with male SVL. The males in the best condition had a SVL at or less than 55 cm.

The hypothesis also predicts that there is an optimal body size for participation in mating aggregations. Males smaller than this size may be in good condition but are not large enough to effectively compete. Males greater than this size would be in poorer condition (or spending more time foraging) which would reduce the number of mating aggregations in which they can participate. I examined the relationship between SVL and the number of mating aggregations in which a male was observed (Figure 2-8). For both years in Barb's marsh, the males that participated in the most aggregations were approximately 55 cm SVL. Thus, these data are consistent with there being an optimal body size for male northern water snakes (approximately 55 cm SVL) for maximizing mating opportunities.

Discussion

I have demonstrated that the size bias between male and female water snakes in this study population is real and not due to an age bias. Males are smaller than females at the same age. An interesting result is that male water snakes rarely exceed 70 cm SVL but can be older than the oldest (and much larger) female in the population. There were more males than females in the total population in Barb's marsh. The ratio of sexually mature individuals (breeding sex ratio) was slightly more male-biased than the population sex ratio. This bias is likely to be a consequence of female water snakes maturing a year later than males; a pattern

Figure 2-8 Relationship between SVL (cm) of each male captured during the breeding season in 1990 (o) (N=42) and 1991 (✕) (N=34) and the number of mating aggregations in which he participated.

exhibited by several snake species with female-biased size dimorphism (Prestt 1971, Feaver 1977, Madsen 1984).

I found that the operational sex ratio of the population was more highly male-biased than the population and breeding sex ratios. Asynchronous breeding by females and the longer duration of mating activity by males relative to females both appear to contribute to the male-bias in the OSR. That there were more males actively mating than females at any given time in the breeding season suggests that males must compete for access to females and therefore, that sexual selection should favour any male characteristic advantageous to mating success. Female-biased size dimorphism in northern water snakes cannot be explained by my first hypothesis that there is no sexual selection acting on males.

Relative tail length did not influence male mating opportunities. It is possible that males in the mating aggregation with the longest tails may monopolize the female and the presence of other males obscures the relationship between long tails and mating success. Long tails could be favoured by sexual selection if a long tail enables a male to copulate more effectively with a female. King (1989) tested this mating advantage hypothesis of why males have longer tails than females. He found no evidence of a mating advantage to greater relative tail length from both an interspecific comparison of 104 snake species and an intraspecific comparison of water snakes. Thus, it is unlikely that male northern water snakes with relatively longer tails were more successful in mating aggregations.

Larger males did participate in more mating aggregations, suggesting selection favouring large size in males. However, male water snakes in this population rarely exceed 70 cm SVL while females can be 15 cm longer. Therefore, there must be another selective pressure which maintains the size difference.

Condition was also an important factor determining the number of mating aggregations in which a male participated. Better condition could be an advantage for gaining mating success if it increases strength or stamina. During copulations, males spasmodically 'jerk', lifting the entwined bodies of the female and other males. If these muscular contractions are necessary to displace competitors, males in better condition may be able to do this more effectively. Another advantage of condition for males could be that males in good condition may require a shorter rest period between matings. Whittier et al. (1985) performed a controlled mating study of the red-sided garter snake (*Thamnophis sirtalis parietalis*), a species which, like the water snake, exhibit 'scramble mating'. Half of the males courted new females within one hour of a previous mating. The other half were unresponsive to females for longer than an hour (Whittier et al. 1985). Perhaps the males in the first group were in better condition than those in the second and thus, able to mate again in a shorter length of time. Other advantages of good condition to male mating success need to be explored.

In my population, there may be no males greater than 70 cm SVL because this body size may be well past the optimal size for maintaining a good condition during the breeding season. I suggest

that there is an optimal body size for males that is a balance between achieving a size large enough to be a viable competitor for mating opportunities and a size small enough that a small weight gain substantially increases the condition of the individual. I suggest that the optimal body size for males in this population is approximately 55 cm SVL. If the optimal size of males is limited by their environment (i.e. food availability), then this value may vary between populations. This could be tested in a comparative study. For example, males of the Lake Erie water snakes attain a SVL up to 86 cm (King 1986). Perhaps they are able to achieve a longer body length than the males in Barb's marsh due to greater prey availability or a larger prey size in Lake Erie. Further studies are required to examine the variance in male reproductive success (e.g. Figure 2-8). As well, further studies are needed to identify the importance of condition to males and their success at mating and whether the advantages of maintaining a good condition does limit the optimal male body size.

Female choice may provide a stronger selective pressure than intra-sexual competition in determining male body size relative to that of females. Females may be actively choosing their mates and not passively accepting the male which succeeds in getting closest to them. If females are choosing a characteristic other than large body size, then an increase in male size would not be expected. Thus, if large males did not achieve the most matings and if large size is gained at a cost, then sexual selection for large body size would not be strong enough to favour a great increase in male size. I attempted to examine the potential influence of female choice of

mates based on body size. Videotaping mating aggregations in the wild was not effective since snakes were intertwined with each other and the branches of the lodge. I also attempted a controlled mate choice experiment in a laboratory setting. However, the snakes in captivity did not show any mating activity. Thus, the influence of female mate choice on size dimorphism between the sexes remains untested.

In summary, I found that, for northern water snakes, there was a male-biased operational sex ratio which leads to male-male competition and sexual selection operating on the males. Good condition and large body size appear to be important to males in order to achieve opportunities to mate. Maintaining good condition may limit the body size of male water snakes and thus, selection may favour a male body size smaller than that of females.

CHAPTER THREE

Multiple Paternity in the Northern Water Snake

Introduction

Sexual selection is the process that favours the evolution of any characteristic that enhances an individual's mating success (Darwin 1871, Trivers 1972). If large size is advantageous for gaining matings, then large males have the highest mating success. Given that females are subject to selective pressures as well, the chief explanation for male-biased size dimorphism in many species is strong sexual selection for large body size in males (Shine 1978, 1979b, Clutton-Brock et al. 1977, Price 1984, LeBoeuf and Reiter 1988).

The strength of sexual selection is positively correlated with the intensity of male-male competition for opportunities to mate (Wade 1979). This intrasexual selection can arise if there are more sexually active males than females (as examined in Chapter Two). However, the strength of intra-sexual competition can also be influenced by the mating behaviour exhibited by the species. In species in which many males in the population have equal opportunities to achieve some reproductive success (i.e. monogamous species), competition between males would not be as intense as if only a few males monopolize most of the matings (i.e. highly polygynous species). If there is high variance in male reproductive success, then male-male competition is intense and sexual selection operating on males is high (Wade 1979, Wade and Arnold 1980). The force of sexual selection is sufficiently strong

that the pattern of observed SSD in a species can be predicted by the mating system exhibited by the species (Shine 1980). In this chapter, I examine the mating behaviour of the northern water snake and its effect on the observed degree and direction of size dimorphism between the sexes.

One explanation for female-biased size dimorphism in a species may be that many males in the population have the opportunity to mate, thus reducing the intensity of male-male competition and the strength of sexual selection on males. Two mating systems in which many males have the opportunity to mate are exhibited in monogamous and promiscuous mating systems. In monogamous species, most males mate with at least one female and the variance in reproductive success between males is reduced. In promiscuous mating systems, the intensity of male-male competition is reduced because each female mates with several males. In northern water snakes, sexually active males outnumber receptive females (Chapter Two). Thus, if females only mate with one male, the competition among males will still be pronounced. Here, I test the hypothesis that female northern water snakes mate with several males, thereby reducing competition among males and thus, decreasing the strength of sexual selection operating on males.

Multiple paternity is well documented in many taxa (e.g., mammals, Birdsall and Nash 1973, Hanken and Sherman 1981; birds, Gowaty and Karlin 1984, Gavin and Bollinger 1985, Evarts and Williams 1987, Gibbs et al. 1990; insects, Ayala et al. 1972, Akin et al. 1984, Levine et al. 1980, Watson 1991; and fish, Avise and Shapiro 1986, Travis et al. 1990), indicating that females of many

species do regularly mate with more than one male. Although multiple paternity is not well documented in snakes, several studies have demonstrated that the offspring of a female snake can be fathered by more than one male. However, most of these studies have involved laboratory-controlled matings of two or three individuals of known genotype or of distinctive phenotype under genetic control (Zweifel and Dessauer 1983, Schuett and Gillingham 1986, Stille et al. 1986). Although these studies demonstrate that multiple paternity is possible, they do not show that it is a natural event, nor do they indicate the extent of its occurrence in the wild. The only direct evidence of multiple paternity in wild populations is from garter snakes (*Thamnophis sirtalis*). Based on a rare melanistic phenotype, Gibson and Falls (1975) demonstrated that females mated more than once. Using protein polymorphisms, Schwartz et al. (1989) found that multiple paternity occurred in 71% of families in two wild populations of garter snakes.

In northern water snakes, highly male-biased (10-12 males per female) mating aggregations have been reported (Tinkle and Liner 1955, this study- Chapter Two). Although the occurrence of multiple mating by females does not require male-biased operational sex ratios, such biases will increase the number of males that a female encounters, and will thereby increase the opportunity for multiple matings. Female garter snakes, which also participate in male-biased aggregations (Gregory 1974), regularly mate with more than one male (Schwartz et al. 1989). Therefore, it seems reasonable to expect that since male-biased breeding sex

ratios are common for northern water snakes as well, then multiple paternity may also be common.

I had two objectives in this chapter. The first was to use field observations to determine the number of different males a female water snake could potentially mate with. The second was to use protein electrophoresis to determine the frequency of multiple paternity in a wild population of this species.

Methods

Field Observations

This study was carried out during the breeding season of 1990. Because I required observations of marked snakes, data for this study were collected simultaneously with the data for Chapter One. Thus, the methods of capturing, marking and observing the snakes were as described in Chapter One. Transects walked through the marsh early in the 1990 season indicated that the majority of mating aggregations occurred on two beaver lodges. Therefore, detailed observations were concentrated at these locations. Between 1 May and the 31 May, I visited the beaver lodges twice a day on every day that the temperature was high enough for the snakes to be active and out on the lodge. During each visit, I recorded which individuals were present at the lodge either by observing the unique dorsal color markings or by capturing unmarked individuals. By early May, most snakes in the marsh had been marked (about 70% of the total captures for the year) and the need to disturb their activity to capture unmarked snakes became minimal.

Since the operational sex ratio was significantly male-biased (Chapter Two), there were many more males available in the population for mating than there were females. However, circumstances may have limited the opportunities females had for mating more than once. To assess the potential for multiple matings by females, I documented all incidences of mating aggregations on the lodges and determined which individuals were participating in each mating aggregation.

Electrophoretic Analysis

To identify polymorphic loci for use in the paternity analysis, I sacrificed five male water snakes collected from sites within the study area but away from the marsh used for the sex ratio study. I sampled tissue from the brain, eye, heart, liver, stomach and muscle and removed 1-2 cm from the tip of the tail. All tissue samples were diluted with an equal volume of homogenizing buffer (0.1 M Tris-HCl, pH 7.0) and frozen immediately at approximately -15°C . Blood was also collected, allowed to clot and centrifuged for 5 min at $20,000 \times g$. The serum was removed, diluted with an equal volume of buffer and frozen. The remaining red blood cells were then also frozen. Tissue samples were transported from the field site on dry ice to ultra-cold storage (-80°C) and electrophoresed within five months.

Extracts for electrophoresis were prepared by homogenizing thawed tissue samples and centrifuging the homogenates at $20,000 \times g$ for 12 min at 4°C . The resulting supernatants were then analyzed by vertical starch gel electrophoresis, following the

procedures described in Philipp et al. (1979). In total, 27 enzyme systems were analyzed using the tissues and serum under a variety of electrophoretic conditions, Tris citrate pH 6.8 (TC), Tris-borate EDTA pH 8.6 (EBT) and Poulik (Murphy et al. 1990) buffer systems. My initial screening process yielded seven polymorphic loci, all of which were expressed in muscle tissue (Table 3-1). Because all seven loci were also expressed in tissue samples obtained from processing the tail tips, I suggest this as a non-destructive sampling technique for future studies.

Paternity Assessment

To obtain broods for paternity analysis, I collected 20 female northern water snakes (45.8-85.1 cm snout-vent length) from several sites within the study area, but again, away from the marsh used for the sex ratio study. Females were collected after the breeding season had ended, between late June and early August, and were held in the laboratory in individual cages measuring 53 by 75 by 46 cm. I fed the snakes at least once a week on live minnows ad libitum. Light bulbs (60W) in each cage provided a direct heat source and were set on a cycle of 14:10h light:dark with the light cycle gradually decreasing through August to 12:12h by the end of September.

After the mothers gave birth to their offspring, muscle tissue samples were obtained from the mothers and all of the offspring (including stillborns). These samples were treated in the same manner as the samples previously described from the males, except that they were stored at -80°C within two days of sampling and all

Table 3-1. Polymorphic loci resolved from muscle tissue of northern water snakes, electrophoretic conditions used, and the number of alleles detected at each locus.

Enzyme (Locus) ^a	E.C.# ^b	Buffer System ^c	# of alleles
Malic enzyme (<i>ME-1*</i>)	1.1.1.40	TC	5
Malic enzyme (<i>ME-2*</i>)	1.1.1.40	TC	2
Isocitric dehydrogenase (<i>IDHP-1*</i>)	1.1.1.42	TC	2
Glucosephosphate Isomerase (<i>GPI-B*</i>)	5.3.1.9	EBT	2
Phosphoglucumutase (<i>PGM-1*</i>)	2.7.5.1	TC/EBT	2
6-Phosphogluconate dehydrogenase (<i>PGD-1*</i>)	1.1.1.44	TC	2
Mannose phosphate isomerase (<i>MPI-1*</i>)	5.3.1.8	TC	2

^a Nomenclature system (Shaklee et al. 1990)

^b Enzyme Commision # - International Union of Biochemistry

^c TC - Tris citrate pH 6.8
EBT - Tris-borate EDTA pH 8.6

were electrophoresed within three months. I determined the genotypes for each individual at each of the seven polymorphic loci.

Snakes were collected under a permit from the Ontario Ministry of Natural Resources. All procedures followed were approved by the Animal Care Committee at Carleton University.

Detecting Multiple Paternity

Since the genotypes at all loci under study were determined for each mother, the observed genotypes of the offspring at these same loci could be used to detect multiple matings by the female in two ways.

1) Direct evidence

For polymorphic loci for which there are three or more detectable alleles, if the genotype of the mother is known, an examination of the array of genotypes observed among the offspring can provide a list of alleles that had to have paternal origin. Since each father can have a maximum of only two alleles at each locus, identification of three or more alleles of definite paternal origin in a brood constitutes direct evidence for multiple matings. For example, at a locus with five alleles, if a mother is heterozygous 2/3 and gives birth to offspring with genotypes: 1/2, 2/3, 2/5, 3/3 and 3/5, then males must have contributed alleles 1, 3 and 5, thus requiring at least two males and thereby providing direct evidence for multiple paternity.

2) Indirect evidence

For polymorphic loci for which only two alleles are detected, indirect evidence for multiple paternity can be obtained by observing significant deviations from the genotype frequencies expected among the offspring if there was a single father. That is, knowing the genotype of the mother, expected offspring genotype frequencies can be generated given any hypothetical genotype for a single father, and tested against observed frequencies using goodness-of-fit statistics or binomial frequencies (Zar 1974). For example, assume that a homozygous mother (1/1) gives birth to offspring with both 1/1 and 1/2 genotypes. If all offspring of this litter were produced from only one father, the father would have to have been heterozygous (1/2). A mating between a heterozygous (1/2) father and a homozygous (1/1) mother would be expected to produce offspring with 1/1 and 1/2 genotypes in a 1:1 ratio. Thus, comparison of observed genotype frequencies in a litter with the expected values identifies those cases for which it is statistically unlikely that all offspring were fertilized by one male. For these cases, I infer that there has been multiple paternity for that litter.

Results

Between 1 May and 1 June 1990, I visited the beaver lodges on 50 occasions to observe mating aggregations. Only once during the 50 visits was a female water snake seen on the lodge with only one male. The highest number of males that a female came in contact with in all mating aggregations in which she participated was 16. On average, 4.15 ± 4.1 different males were in all mating

aggregations for any particular female. Thus, breeding female water snakes appear to have ample opportunity to mate with more than one male.

Of the eight different females observed in mating aggregations during these 50 visits to the lodges, five were seen in more than one aggregation. In no case were successive aggregations involving a particular female comprised of exactly the same group of males. Furthermore, no male was present in all the aggregations observed for a particular female. Therefore, if mating occurred in every aggregation I observed, at least 62% (5/8) of the females must have mated with more than one male.

Of the 20 captive female water snakes, 14 gave birth to a total of 322 offspring (including four stillborns) between 5 and 20 September 1990. Litter size ranged from eight to 37 individuals (including stillborns) with a mean of 22.6 ± 8.3 young per litter. The remaining six females did not give birth for reasons that remain unknown (i.e. these females were not distinguishable from the other females based on body size or date of capture).

I detected multiple paternity in 12 of the 14 litters. Examination of the distribution of the five alleles at the *ME-1* * locus among the offspring of each litter provided direct evidence that eight of the 14 litters were fathered by more than one male (Table 3-2). Of the remaining six litters, comparison of the observed offspring genotypic frequencies with those expected from simple Mendelian values provided indirect evidence that multiple matings occurred in four additional litters (Table 3-3). For all litters in which multiple paternity was detected at the *ME-1* *

Table 3-2. Evidence of multiple paternity based on alleles detected at the *ME-1** locus in offspring of litters of northern water snakes

Litter #	Litter Size	Female Genotype	Offspring Genotypes	N	Paternal Allelic Contributions	Estimated # of males
1	25	2/3	3/5	8	1,3,5	≥ 2
			1/2	2		
			3/3	7		
			2/5	6		
			2/3	3		
2	13	3/5	1/3	3	1,5	1
			5/5	6		
			1/5	3		
			3/5	1		
3	29	3/5	1/3	6	1,2,3,5	≥ 2
			1/5	8		
			3/5	8		
			5/5	3		
			3/3	3		
4	31	1/3	2/3	1	3,5	1
			1/5	8		
			3/5	7		
			3/3	10		
5	30	5/5	1/3	6	1,3,5	≥ 2
			1/5	15		
			3/5	11		
6	19	1/5	5/5	4	1,3,5	≥ 2
			1/1	6		
			3/5	2		
			5/5	1		
			1/3	1		
7	16	2/2	1/5	9	3,5	1
			2/5	7		
			2/3	9		
8	16	1/5	1/5	7	5	1
			5/5	9		
9	17	1/5	4/5	7	2,4,5	≥ 2
			1/2	8		
			5/5	1		
			1/5	1		
10	24	3/5	1/5	8	1,2,4,5	≥ 2
			5/5	3		
			3/5	9		
			3/4	3		
11	22	5/5	2/5	1	3	1
			3/5	22		

12	37	2/3	3/3	12	1,2,3,5	≥ 2
			2/3	17		
			2/5	2		
			1/2	2		
			1/3	1		
			2/2	2		
13	31	3/3	3/5	1	1,3,4,5	≥ 2
			3/3	14		
			1/3	12		
			3/4	1		
			3/5	4		
14	8	5/5	3/5	8	3	1

Table 3-3. Offspring genotypic proportions within litters of northern water snakes. Significant deviations from those expected based on Mendelian principles of heredity for one father indicate incidences of multiple paternity. Significance tests by goodness of fit or binomial frequencies (Significance levels are * $p=.05$, ** $p=.01$, *** $p<.001$).

Litter # (N)	Locus (Maternal genotype) Offspring genotype frequency						# of males
	ME-2*	IDHP-1*	GPI-B*	PGM-1*	PGD-1*	MPI-1*	
1 (25)	(1/1) ** 1/1 .80 1/2 .20	(2/2) 1/2 .68 2/2 .32	(1/2) 1/1 .36 1/2 .52 2/2 .12	(1/1) 1/2 1.0	(2/2) 2/2 1.0	(2/2) 2/2 1.0	≥2
2 (13)	(2/2) 2/2 1.0	(1/2) 1/2 .69 2/2 .31	(1/2) 1/1 .30 1/2 .40 2/2 .30	(1/1) ** 1/1 .92 1/2 .08	(1/2) 1/1 .38 1/2 .62	(1/2) 1/1 .08 1/2 .46 2/2 .46	≥2
3 (29)	(1/1) ** 1/1 .24 1/2 .76	(2/2) 2/2 1.0	(1/2) ** 1/1 .21 1/2 .75 2/2 .04	(1/1) 1/1 1.0	(2/2) 2/2 1.0	(2/2) *** 1/2 .17 2/2 .83	≥2
4 (31)	(2/2) 1/2 .45 2/2 .55	(2/2) 2/2 1.0	(1/1) 1/1 .62 1/2 .38	(1/1) 1/1 .55 1/2 .45	(2/2) 2/2 1.0	(2/2) ** 1/2 .26 2/2 .74	≥2
5 (30)	(1/2) 1/1 .16 1/2 .44 2/2 .40	(2/2) 2/2 1.0	(1/2) 1/1 .23 1/2 .43 2/2 .33	(1/1) *** 1/1 .86 1/2 .14	(2/2) 2/2 1.0	(2/2) 1/2 .50 2/2 .50	≥2
6 (19)	(1/1) 1/1 .42 1/2 .58	(2/2) 1/2 .32 2/2 .68	(1/1) ** 1/1 .84 1/2 .16	(1/1) 1/1 1.0	(2/2) 2/2 1.0	(2/2) 2/2 1.0	≥2
7 (16)	(2/2) 1/2 .45 2/2 .55	(1/2) 1/1 .50 1/2 .50	(1/1) 1/1 1.0	(1/1) 1/1 1.0	(2/2) 2/2 1.0	(2/2) 2/2 1.0	1
8 (16)	(1/2) 1/1 .38 1/2 .31 2/2 .31	(1/2) 1/1 .32 1/2 .50 2/2 .18	(1/1) 1/1 .5 1/2 .5	(1/1) 1/1 1.0	(2/2) 2/2 1.0	(2/2) 1/2 .50 2/2 .50	1
9 (17)	(1/2) ** 1/2 .83 2/2 .17	(1/2) 1/1 .12 1/2 .41 2/2 .47	(1/2) ** 1/1 .58 1/2 .35 2/2 .07	(1/1) 1/1 1.0	(2/2) 2/2 1.0	(1/2) 1/2 .41 2/2 .59	≥2

10	(1/2) *	(1/2) *	(1/2) **	(1/2)	(1/2)	(2/2) ***	
(24)	1/1 .46	1/1 .08	1/1 .50	1/1 .17	1/1 .46	1/2 .12	≥2
	1/2 .50	1/2 .46	1/2 .42	1/2 .58	1/2 .38	2/2 .88	
	2/2 .04	2/2 .46	2/2 .08	2/2 .25	2/2 .17		
11	(1/2)	(1/2)	(1/2)	(1/2)	(2/2)	(1/2) **	
(22)	1/1 .24	1/1 .10	1/1 .43	1/1 .38	2/2 1.0	1/1 .05	≥2
	1/2 .43	1/2 .52	1/2 .47	1/2 .47		1/2 .43	
	2/2 .33	2/2 .38	2/2 .10	2/2 .15		2/2 .52	
12	(1/2)	(2/2)	(1/2)***	(1/1)	(2/2)	(2/2) ***	
(37)	1/1 .19	1/2 .35	1/1 .51	1/1 1.0	2/2 1.0	1/2 .22	≥2
	1/2 .46	2/2 .65	1/2 .46			2/2 .78	
	2/2 .35		2/2 .03				
13	(1/2) *	(2/2)	(1/1)	(1/1)***	(2/2)	(2/2) *	
(31)	1/1 .38	2/2 1.0	1/1 .55	1/1 .87	2/2 1.0	1/2 .13	≥2
	1/2 .26		1/2 .45	1/2 .13		2/2 .87	
	2/2 .36						
14 (8)	(1/2)	(1/2)	(1/1) *	(1/2)	(1/2)	(2/2)	
	1/2 .75	1/1 .25	1/1 .13	1/1 .38	1/2 .63	2/2 1.0	≥2
	2/2 .25	1/2 .50	1/2 .87	1/2 .62	2/2 .37		
		2/2 .25					

locus, at least one other locus also gave supporting evidence that the female had mated more than once (Table 3-3), demonstrating the effectiveness of detecting multiple paternity using the indirect method. Thus, although 57.1% (8/14) of the litters of northern water snakes in this study were unequivocally multiply fathered, a value of 85.7% (12/14) may be a more accurate estimate. In fact, because some males will share genotypes at all the loci I examined, I cannot exclude the possibility that multiple paternity occurred in all broods.

Discussion

I have demonstrated that highly male-biased operational sex ratios exist within mating aggregations of northern water snakes. This bias, together with the observed participation of individual females in aggregations involving different males, indicated that female water snakes have ample opportunity to mate with more than one male. I have also demonstrated that the estimated occurrence of multiple paternity within the study population of northern water snakes was 85.7% and may have been higher.

There were fewer mating aggregations in 1991 than in 1990 throughout the marsh (1990 N=50; 1991 N=21). As well, of those females observed actively mating, fewer were observed in more than one mating aggregations in 1991 than the year previously (3/8 in 1991, 5/8 in 1990). This difference between the years suggest that the frequency of multiple paternity may be less in 1991 than 85.7% observed in 1990. Thus, caution is necessary when predicting the frequency of occurrence of multiple paternity. An electrophoretic

paternity analysis of water snake broods would need to be repeated over several years to get an accurate estimate of the occurrence of multiple paternity in a population of northern water snakes.

The fact that females mate with more than one male would seem to reduce the variance in male reproductive success in the population. However, since I was unable to determine which males actually successfully sire offspring, I cannot rule out the possibility that it is a small subset of males that are successful at mating with each female. Thus, the competition between males for mating with females could still be high despite the occurrence of multiple paternity. In a controlled mating study of the red-sided garter snake, Whittier et al. (1985) found that only 29% of the males exposed to females actually mated and that only half of these males mated more than once. A small percentage of the male population achieved most of the matings. Thus, although a female water snake mates with several males, only a few males in the population may be successful. Competition between males would still be intense and sexual selection pressures influencing the sexual size differences. However, this study shows that several males do achieve matings with a single female and multiple paternity occurs. Together, these results indicate that the variance in male reproductive success is not as high and competition not as intense as if there was single paternity.

For most species of animals, males can increase their reproductive success with successive matings (Trivers 1972). However, for species in which a single mating is sufficient to fertilize all of a female's offspring, multiple matings have no such

obvious benefit to the female (Halliday and Arnold 1986). In some species, forced copulation may be the cause of multiple paternity (e.g. mallards, Evarts and Williams 1989). In other species, males cannot force copulations and multiple matings are probably due to female choosing to mate with several males.

In snakes, forced copulations are unlikely to occur since the male hemipenes have neither a baculum or hydrostatic pressure which would be necessary to force apart the scutes covering the female's vent (Devine 1984). During courtship, female snakes must lift their tail and expose their cloaca to permit intromission and can terminate mating at any stage during copulation (Perry-Richardson et al. 1990). Thus, the observed multiple paternity in water snakes seems likely to be a result of female choice for mating with several males.

Several advantages for multiple matings by females have been suggested. Females may mate with several males to insure against the possibility that one male is sterile (Gibson and Jewel 1982), or if, contrary to what has been assumed, a single ejaculate is not sufficient to fertilize an entire brood (Gromko et al. 1984). In addition, acceptance of matings from several males may guard against possible genetic defects (Halliday and Arnold 1986) and/or produce more genetically diverse offspring (Williams 1975). Females could also solicit multiple matings to promote sperm competition (Parker 1970). Alternatively, females may mate repeatedly simply because high mating frequency has been selected for in males and is manifested in females because of genetic

correlation between the sexes for mating behaviour (Halliday and Arnold 1986).

Whatever the advantages are to the female of mating with more than one male, they must outweigh any potential costs or disadvantages. Courtship and copulation are known to put participants at risk from predation (Ross and Crews 1977). For female water snakes, either the risk of predation during mating aggregations is small so that females with an abundance of available males have little cost to mating more than once, or the potential gain in reproductive success through multiple inseminations compensates for the additional risk of participating in several matings.

Even if multiple mating in snakes is correlated with a bias in breeding sex ratio, we must still explain why females choose to mate with more than one male when given the opportunity. There are several obvious questions with which to start. For example, do females mate indiscriminately and rely on sperm competition to determine the success of the males or are females selective about their choice of mates? If females are selective, which male traits are correlated with success? Answers to these questions will require the use of genetic markers to assign paternity to water snake broods as is now being done with birds (Weatherhead and Montgomerie 1991).

CHAPTER FOUR

Natural Selection on Body Size of Northern Water Snakes

Introduction

Some degree of morphological, physiological and behavioural variation exists among individuals of all species. Certain individuals are better able to survive and reproduce in particular environments than others. If some of the variation that enhances an individual's ability to survive or reproduce is heritable, then genes producing the successful variants will become more abundant in successive generations. It is this process of natural selection that was the central mechanism for evolution proposed by Darwin (1859). Unlike sexual selection, which favours traits beneficial to mating success but potentially detrimental to survival (Darwin 1871), natural selection favours traits beneficial to survival and increased reproductive success. In this chapter, I test hypotheses regarding natural selection and the pattern of SSD in northern water snakes.

One explanation of female-biased size dimorphism in northern water snakes may be that selection has favoured large female body size because large size increases fecundity (Darwin 1871, Ralls 1976). For many animal species, larger females produce more offspring (insects, Wiklund and Karlsson 1988) or larger offspring (birds, Parsons 1970, insects, O'Neill 1985) than smaller females. Such a relationship has also been found in several snake species (Fitch 1981, Semlitsch and Gibbons 1982, Ford and Killebrew 1983, Madsen 1983, King 1986, Ford and Seigel 1989). In addition to a fecundity advantage, larger female snakes allocate less reproductive

effort to each progeny than do smaller females (Lemen and Voris 1987). All female snakes allocate an approximately equal proportion of their total energy to their reproductive effort, regardless of their body size (Semlitsch and Gibbons 1982, Ford and Killebrew 1983, Seigel et al. 1986). However, since a larger female has more offspring than a smaller female, then a larger female would be investing less of her reproductive effort to each offspring than would a smaller female. My first objective was to examine if larger female northern water snakes have increased fecundity and if they allocate less reproductive effort per offspring than smaller females.

Another natural selection pressure that may influence the degree of SSD exhibited by a species is differential mortality between the sexes. Reproductive activities differ between the sexes of most animal species and may result in differential vulnerability to predation. Thus, each sex may have different traits or different variations of a trait (e.g. different body size) selected to minimize their risk of predation. Males of many species are more conspicuous than females during the breeding season as they search for or try to attract females (e.g. anuran mating calls, Arak 1988). In many species, males are at great risk while competing for females either by inflicting injuries on each other while fighting (e.g. red deer *Cervus elaphus*, Clutton-Brock et al. 1979) or by becoming more conspicuous to and less aware of predators (e.g. adders *Vipera berus*, Madsen 1983). Mating aggregations, such as those exhibited by northern water snakes and garter snakes (*Thamnophis* sp.), are also conspicuous and potentially put the

participants at risk (Ross and Crews 1977). Since males usually outnumber the females in mating aggregations, males are more likely to be at risk from predation than females while participating. If male northern water snakes suffer high mortality from predation due to their conspicuousness during the breeding season both in searching for females and by participating in mating aggregations, small size could be selected due to its possible advantage in escaping predators. An alternative consequence of a high risk of predation for males during the breeding season may be that males do not live long enough to attain a large body size. However, since the age distribution for males and females was approximately equal in the population in Barb's marsh (see Chapter Two), the first possibility remains the more likely option. My second objective was two fold - first, to determine if males are more conspicuous than females during the breeding season and second, to examine if male participation in mating aggregations potentially increases their risk of predation.

Female reproductive activity could also increase the risk of predation for females. Females must ensure that the developing embryos are incubated at the optimal temperature. Unlike mammals, reptiles, as poikilotherms, must achieve this behaviourally rather than physiologically. Oviparous species may lay their eggs in warm substrate for proper development (e.g. turtles, Leviton 1971) while females of viviparous species, such as water snakes, must elevate their own internal body temperature. Gravid female lizards have been found to bask more than males and non-gravid females (Shine 1980). As well, gravid female lizards and snakes have reduced

greater rate than males due to the increasing weight of the young they are carrying. However, the last weight measurement was taken on June 20 probably not late enough in the season for such weight difference to be apparent.

Discussion

Larger female northern water snakes have more offspring and expend less reproductive effort per progeny than do smaller females. These results appear to support the fecundity advantage hypothesis as an explanation of female-biased size dimorphism. However, there are several arguments that indicate that the fecundity advantage hypothesis alone is not sufficient to explain why females are the larger sex. First, there must be some selective pressure preventing males from attaining the large size of the females due to genetic correlation (Lande 1980). Second, selective pressures favouring large mothers seem to apply equally to mammalian species exhibiting male-biased size dimorphism as to mammalian species exhibiting female-biased size dimorphism (Ralls 1976). Third, females could increase fecundity with an increase in body size only if there is enough available energy to support the additional offspring (Shine 1980). These arguments indicate that, although there seems to be a strong relationship between female body size and increased reproductive success, this hypothesis alone does not explain female-biased size dimorphism.

Male water snakes appear to be potentially more vulnerable to predation due to their participation in mating aggregations and

mobility compared with males and non-gravid females which limits their ability to capture prey and escape predation (Shine 1980, Bauwens and Thoen 1981, Seigel et al. 1987, Van Damme et al. 1989). This is a particular problem for species, such as northern water snakes, that require speed and agility to pursue prey and escape predators (Magnhagen 1991). If larger individuals can swim faster than smaller ones before the burden of carrying young, then large body size may be advantageous when gravid. Larger individuals may also have fewer predators than smaller snakes, an additional advantage when they have reduced mobility. If gravid females have a higher potential mortality due to the need to bask and having limited mobility and if large size is advantageous to survival and reproductive success, there would be strong selection for large body size in females. My third objective was to determine if female water snakes do bask more often and are more conspicuous overall than males after the breeding season.

Participation in mating activities can also be costly apart from the increased risk of predation. Males of many animal species forgo feeding during the mating season and expend enormous amounts of energy in attempting to secure as many mates as possible (e.g. red deer, Clutton-Brock et al. 1982, elephant seals, Cox and LeBoeuf 1977). If male northern water snakes are not foraging during the breeding season and are expending energy to search for females and to compete with other males in the mating aggregations, then there could be an energetic constraint on growth in males. My fourth objective was to determine if activities during

the breeding season are sufficiently energetically costly to males that their annual growth could be restricted.

Methods

To examine if there is a fecundity advantage for large females, I gathered data from the broods of 14 female water snakes that gave birth in captivity in 1990 (Chapter Three). Each female was weighed within 12 hours of giving birth to determine the post-parturition weight (PPW) (to the nearest g). I measured total length, vent to tail tip length and weight of each neonate.

To examine the reproductive effort per offspring, I first calculated the proportion of each female's body mass allocated to reproduction. Relative clutch mass (RCM) (clutch mass/ female body weight after birth) is an operational measure of a female's reproductive effort (Tinkle 1972 but see Cueller 1984 for discussion). Dividing the RCM by the clutch size gives the reproductive effort per progeny for each female.

I examined whether males are more conspicuous than females during the breeding season by gathering data on the relative number of sightings of male and female water snakes in Barb's marsh. I defined the breeding season as occurring between 30 April and 3 June 1990 and between 30 April and 24 May 1991 - the dates of the first and last observations of mating aggregations in each year. Before the breeding season started, I visited the marsh daily and captured as many snakes as possible. Methods for capturing, measuring and marking snakes were as described in Chapter Two. I also examined each snake captured for scars and apparent injuries.

During the breeding season, I walked transects approximately 3 m apart through the whole marsh at least three times a week in 1990 and at least once a day in 1991. While walking the transects, I recorded sightings of all snakes either by noting their paint markings or by attempting to capture any unmarked individuals. I recorded the location in the marsh and the habitat for each sighting. I defined three habitat types in which snakes were observed; 1) water, 2) lodge (one of the two beaver lodges) and 3) branches which included all other locations in the marsh out of the water such as, willow branches, stumps and grass clumps.

If a particular snake was observed more than once during a given visit to the marsh, only the first observation was used in analyses. For any given visit, there were a few snakes (range 0- 4) that I could not identify properly or unmarked snakes that I could not catch. Since the sex of these individuals was not known, these snakes were not included in the analyses. Because the visits were at least one day apart, the snakes could move freely and I rarely disturbed the activity of the snakes, I used each visit to the marsh as an independent assessment of relative numbers of sightings of males and females.

Only sightings of sexually mature snakes were used in these analyses. Sexual maturity was defined in Chapter Two for males as those individuals with a SVL greater than 42.5 cm and for females as those individuals with a SVL greater than 58.7 cm. I compared the sex ratios of sightings with those expected based on captures of sexually mature individuals (Chi-square test, Zar 1984). Sex ratios of snakes sighted during the breeding season were compared with

the sex ratio of only those sexually mature individuals caught during the breeding season. Sex ratios of sightings after the breeding season were tested against the sex ratio of all captures of sexually mature individuals.

The second part of assessing the potential risk of predation for males was to examine whether participation in mating aggregations is risky for males. I performed an approach experiment on 18 of the total of 51 mating aggregations encountered in 1990 and 12 of the 21 mating aggregations observed in 1991. When I first noticed a mating aggregation, I stopped far enough away to not disturb the snakes activity, while I recorded the identity of all participants. For the mating aggregations on which I performed the approach experiment, I then walked towards the mating aggregation at a steady pace and noted which individual left the mating aggregation first and the distance I was from the group when it broke apart. Several females in both years were observed in multiple mating aggregations that were not disturbed. Thus, female participation in multiple aggregations was not due to the fact that I disturbed some mating aggregations. For comparison, I also performed the approach experiment on several occasions (N=4 in both years) when many snakes were basking near one another but were not mating.

My third objective was to determine if females are more conspicuous than males after the breeding season. I gathered data on the relative number of sightings of male and female snakes in the marsh after the breeding season, continuing the same transects as for the breeding season. I defined the start of the post-breeding

season as the day after I observed the last mating aggregation. I gathered data until 06 July 1990 and 26 June 1991, approximately one month after the end of the breeding season.

To investigate whether the reproductive activities of males during the breeding season are energetically costly, I examined the weight of specific individuals through the breeding and post-breeding seasons in 1991. I weighed snakes captured opportunistically at approximately weekly intervals. The weighing was completed in less than a minute in the field and the snakes were released immediately to minimize disturbance of their activity. I calculated the weight change of individuals per day by dividing the difference in weight measured during the breeding season by the number of days between measurements. I also did this for weight measurements after the breeding season. I compared the average weight change of males during and after the breeding season and between males and females both during and after the breeding season by an analysis of variance using body size (SVL) as a covariate.

Results

Fecundity Advantage Hypothesis

A total of 322 young were born to 14 females (Table 4-1). Larger (SVL) females produced heavier clutches ($r^2=.77$, $F = 42.16$, $df = 12,1$, $p < .0001$) than smaller females (Figure 4-1). Since a female could potentially have many small offspring or few large offspring, I determined how females allocated their energy to the offspring. Clutch size increased with female SVL ($r^2=.63$, $F=20.5$, $df=12,1$,

Table 4-1 Summary of reproductive activity of female northern water snakes (N=14) including snout-vent length (SVL), post-parturition weight (PPW) and relative clutch mass (RCM) (clutch mass/ PPW). Mean values are given $\pm$ one standard deviation (SD).

	$\bar{X}$	SD	Range
Female SVL (cm)	76.8	4.7	68.5-86.0
Female PPW (g)	294.6	54.7	233-379
Clutch size	22.7	8.3	8-36
Clutch mass (g)	86.5	29.0	25.5-143.0
Ave young SVL (cm)	16.7	.89	15.4-17.9
RCM	.286	.07	.11-.38

Figure 4-1 Clutch mass (g) relative to female body size (snout-vent length - SVL) (N=14).

$p=.0007$) but the average neonate weight in the clutch was not correlated with female SVL ($r^2=.01$, $F=.117$, $df=12,1$, $p=.73$). Thus, larger females have more young than smaller females but all females produce young of approximately the same size.

Female water snake reproductive effort (RCM) was correlated with body size (SVL) ($F=6.04$, $df=12,1$, $p=.012$). However, this significance was entirely attributable to one female. This female allocated a significantly smaller proportion of her body weight to her offspring than did the other females (RCM = .11 Dixon's test for outliers, Sokal and Rolf 1981). When this female was removed from the analysis, reproductive effort was not correlated with body size ($r^2=.13$, $F=1.72$, $df=11,1$, $p=.21$), a result similar to that obtained for other snake species. Thus, larger females allocated less reproductive effort to each progeny than did smaller females ($r^2=.39$, $F=7.09$, $df=11,1$, $p=.02$) (Figure 4-2). Increased body size appears to allow females to produce more young and with lower reproductive effort per young.

Sightings During Breeding Season

Males were more likely than females to be sighted during the breeding season in 1990 and in both years combined (Table 4-2). The sex ratio of sightings in 1991 was male-biased, although not significantly more so than the sex ratio of all sexually mature individuals. Males were sighted more often than females in the water and on the lodges during the breeding season in 1990. Since males and females use different habitats during the breeding season,

Figure 4-2 Reproductive effort per progeny relative to female body size (SVL) (N=13). Reproductive effort measured as relative clutch mass (RCM) or total clutch mass / female weight after parturition.

Table 4-2 Sex ratios (Male: Female) of sightings and habitat use of sexually mature northern water snakes.

	1990		1991		Combined	
	N	MF	N	MF	N	MF
During breeding season						
Captures	64	1.9:1	54	2:1	118	1.9:1
Sightings ^a	472	2.3:1 *	395	1.9:1 NS	867	2.2:1 *
Branch ^a	105	1:1.69 ***	172	1.04:1 *	277	1:1.18 ***
Lodge ^a	316	3.03:1 *	193	2.17:1 NS	509	2.9:1 *
Water ^a	51	4.1:1 *	37	3.6:1 NS	88	3.8:1 *
Total captures	75	1.77:1	60	2:1	135	1.9:1
Post breeding season						
Sightings ^b	279	1.23:1 NS	201	1.71:1 NS	480	1.4:1 NS
Branch ^b	83	1:1.67 **	86	1.04:1 NS	169	1:1.25 ***
Lodge ^b	163	2.01:1 NS	91	2.64:1 NS	254	2.2:1 NS
Water ^b	33	1:1.35 *	24	2.42:1 NS	57	1.19:1 NS

^a Sex ratios tested against expected values based on captures during breeding season

^b Sex ratios tested against expected values based on total captures

Levels of significance of chi-square test * $p < .05$, ** $p < .01$, *** $p < .0001$ NS - non significant

the sexes would be potentially differentially vulnerable to predation.

Approach Experiments

A typical scenario of the approach experiment was as follows. As I approached a mating aggregation, the female would suddenly lift her head and resist the pushing by the males. The males would continue to move about her and wrap their tail around hers. As I approached closer, the female would leave the group of snakes in a sudden motion and swim quickly out of sight. A female never returned to the lodge while I remained there. After the female was gone, the males stayed in the area apparently oblivious to my presence. They moved ceaselessly and randomly around the area where the female had been. The males were very easy to catch when in this state, sometimes crawling over my boots as I stood on the lodge.

The mating aggregations I approached varied in size from two snakes (one male and one female) to 13 snakes (12 males and one female). There was only ever one female in a mating aggregation and males, on average, outnumbered the females (Number of males: 1990 $\bar{x}=2.5$, $SD=2.6$, $N=18$; 1991 $\bar{x}=2.8$, $SD=1.9$, $N=12$). Thus, by chance alone, males would be most likely to be first to leave mating aggregations. However, a female was the first to leave all but two of the mating aggregations I approached during both years (1990 $N=18$, 1991 $N=12$). The approach distance at which the females left ranged from 1-10 m and did not vary with size of the mating

aggregation in which the female was participating ($F= 14.5$, $df=31$, $p=.21$).

Of the four non-mating aggregations I observed in each year, males outnumbered the females by a ratio slightly greater than 2:1 (Number of males 1990 $\bar{x}=2.75$, $SD=2.5$, $N=4$; 1991 $\bar{x}=2.5$, $SD=1.7$, $N=4$). As expected based on chance alone, a male was the first to leave all but one of the non-mating aggregations observed in 1990. Males and females were equally likely to be the first one to leave in the non-mating aggregations observed in 1991. Tolerance of approach distance appears to differ between individuals irrespective of sex when they are not engaged in mating activity. For example, some females stayed on the lodge even when I got onto it while other females would leave when I was about 4 metres away. Unfortunately, the data were insufficient to examine if tolerance of approach behaviour of each individual was consistent daily or depended on some factor such as the thermal and nutritional state of the individual.

If males are more vulnerable to predation than females due to the combined effect of an increased conspicuousness during the breeding season and their increased vulnerability in mating aggregations, then I would expect a higher incidence of injury in males than females. I used data on the presence of scars and stub tails for snakes captured in Barb's marsh during the two years of my study and during a study performed two years previous to mine. The majority of all snakes observed with scars or injuries were females and the proportion of females of the total number of individuals with scars increased with each age group (Figure 4-3).

Figure 4-3 Percentage of each sex of the total number of individuals that have scars or injuries in each age class (year). Females are represented by ▲ and males by o.

Sightings After the Breeding Season

Females bask on branches more than do males both during and after the breeding season. This may increase their vulnerability to predation if snakes on branches are more conspicuous to predators than snakes in other habitats. After the breeding season, females were not more likely than males to be sighted overall. Thus, there does not seem to be strong evidence that females are potentially more vulnerable to predation than males based on relative frequency of sightings.

Energetic Constraints

There was no difference in the daily weight change of males during and after the breeding season (Table 4-3), contrary to what would be expected if the breeding season was energetically costly to males. There was no relationship between the number of mating aggregations in which a male was observed and his weight change during the breeding season ($F=.159$, $df= 22$, $p=.52$). Thus, participation in mating aggregations did not appear to influence male weight gain or loss.

Females gained significantly more weight daily per gram of body weight than did males during the breeding season (Table 4-3). This result suggests that males were either not feeding as often as females or that the energy from feeding was not allocated to weight gain for males. There was no difference between the sexes in weight change in the month following the breeding season (Table 4-3). Gravid females would be expected to increase weight at a

Table 4-3 Comparison of incidences of scars and injuries on male and female northern water snakes. Tests of significance against the population sex ratio (M:F 1.4:1).

Injury	N	M:F	X ²	p value
Head	37	1:1.46	14.32	.038
Body	4	0:4	5.64	.018
Tail	19	1.7:1	.1	.75
Total	58	1:1.15	2.84	.093

greater rate than males due to the increasing weight of the young they are carrying. However, the last weight measurement was taken on June 20 probably not late enough in the season for such weight difference to be apparent.

Discussion

Larger female northern water snakes have more offspring and expend less reproductive effort per progeny than do smaller females. These results appear to support the fecundity advantage hypothesis as an explanation of female-biased size dimorphism. However, there are several arguments that indicate that the fecundity advantage hypothesis alone is not sufficient to explain why females are the larger sex. First, there must be some selective pressure preventing males from attaining the large size of the females due to genetic correlation (Lande 1980). Second, selective pressures favouring large mothers seem to apply equally to mammalian species exhibiting male-biased size dimorphism as to mammalian species exhibiting female-biased size dimorphism (Ralls 1976). Third, females could increase fecundity with an increase in body size only if there is enough available energy to support the additional offspring (Shine 1980). These arguments indicate that, although there seems to be a strong relationship between female body size and increased reproductive success, this hypothesis alone does not explain female-biased size dimorphism.

Male water snakes appear to be potentially more vulnerable to predation due to their participation in mating aggregations and

conspicuousness during the breeding season. Female water snakes bask on branches more than do males potentially increasing their vulnerability to predation. Further studies will be necessary to examine if differential risk of predation between the sexes due to their reproductive activities results in strong selection on the relative body size of male and female water snakes.

Males appeared to have reduced energy intake or a higher energy expenditure during the breeding season compared with females. However, unlike males in many other species, there was not a significant decline in weight at the end of the breeding season. King (1986) reported that females appear to feed over a longer portion of the active season than do males and that, although weight gain occurs throughout the season in adult females, it is restricted to mid-summer for males. A possible explanation of why I did not observe a difference in weight gain in males during and after the breeding season may be that individuals were not followed far enough into the summer to observe a substantial weight change. Thus, I was not able to accurately assess the potential contribution of energetic constraints as a factor selecting for small body size in males.

Several other natural selection hypotheses have been proposed to contribute to the explanation of the degree and direction of SSD in a species. The degree of SSD could be a result of the reduction of intersexual competition through partitioning of ecological resources (Schoener 1966, Selander 1972, Ralls 1976, Woolbright 1983, Carothers 1984, Shine 1989a, 1991). As well, several life history traits, including SSD, have been found to vary significantly between

geographically distinct populations within species of lizards and snakes (Tinkle and Ballinger 1972, Fitch 1981, Dunham 1982).

Behavioural differences between geographically separate populations would alter selection pressures and would influence the relative sizes of the sexes of a species.

In summary, greater fecundity advantage and reduced reproductive effort per young may be strong advantages of large female body size. As well, my data indicate that there may be a potential difference in the risk of predation between the sexes which may select for different body size optima of males and females.

FINAL CONCLUSIONS

Chapter One: Snakes follow the general allometric trend that males are relatively larger than females in larger species. Sexual size dimorphism differs between snake families. However, allometry and phylogenetic constraints explain only a small portion of the variation in the patterns of SSD observed in snakes. Adaptation to ecological conditions likely contributes to the difference in sizes between the sexes.

Chapter Two: Female northern water snakes are larger than males at the same age. Because the operational sex ratio is male-biased, body size in male water snakes is probably influenced by sexual selection. Although large size may confer an advantage to males in competition in mating aggregations, good condition may be more important in increasing male reproductive success. Thus, the optimal body size for male water snakes may be limited by the advantages of maintaining good condition. Further studies are required to investigate this.

Chapter Three: I observed female northern water snakes participating in multiple mating aggregations. Protein electrophoretic analysis of broods of females mated in the wild indicated that, for the majority of the females, more than one male sires her offspring. Thus, competition between males may be less intense than if only one male fathered the entire brood of most females. However, I was unable to rule out the possibility that a

few males may be dominating matings in a population. Studies which assign paternity by DNA fingerprinting are needed to examine this more closely.

Chapter Four: Natural selection also influences the body sizes of the two sexes. Larger females have more offspring and expend less reproductive effort per progeny; thus favouring large female size. Males appear to have a potentially higher risk of predation than females during the breeding season due to their relatively greater conspicuousness and increased vulnerability in mating aggregations. Thus, the influence of differential predation risk on the relative sizes of the sexes needs to be examined further.

I have found evidence that selection to increase reproductive success influences the body size of both sexes. The optimal body size for females may be largely due to natural selection favouring increased fecundity. The optimal body size for males may be smaller than females due to sexual selection and the reproductive advantages of limiting body size to maintain good condition. In this thesis, I have documented several factors influencing the relative body size of males and female water snakes and have identified further avenues of research.

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