12-2000

Plasticity of morphology, chemoreception, and predatory behavior in garter snakes (Thamnophis Sirtalis)

Mark Andrew Krause

To the Graduate Council:

I am submitting herewith a dissertation written by Mark Andrew Krause entitled "Plasticity of morphology, chemoreception, and predatory behavior in garter snakes (Thamnophis Sirtalis)." I have examined the final electronic copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Psychology.

Gordon M. Burghardt, Major Professor

We have read this dissertation and recommend its acceptance:

Richard Saudargas, Christine Boake, Gary McCracken, James Lawler

Accepted for the Council:

Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

To the Graduate Council:

I am submitting a dissertation written by Mark A. Krause entitled "Plasticity of Morphology, Chemoreception, and Predatory Behavior in Garter Snakes (*Thamnophis sirtalis*)." I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Psychology.

Gordon M. Burghardt
Major Professor

We have read this dissertation
and recommend its acceptance:

Christine R. Boake

Accepted for the Council:

Interim Vice Provost and
Dean of the Graduate School

PLASTICITY OF MORPHOLOGY, CHEMORECEPTION, AND PREDATORY BEHAVIOR IN GARTER SNAKES (*THAMNOPHIS SIRTALIS*)

A Dissertation
Presented for the
Doctor of Philosophy
Degree
The University of Tennessee, Knoxville

Mark A. Krause
December, 2000

DEDICATION

This dissertation is dedicated to my family

James Krause

Ruthanne Krause

&

Matt Krause

ACKNOWLEDGMENTS

Many people deserve a lot of credit for making the completion of this dissertation possible. First of all, I thank Dr. Gordon M. Burghardt for helping to make the study of animal behavior my passion. His support, guidance, and encouragement made this a great learning experience. Dr. Richard Saudargas supported me in developing and writing this dissertation, and was instrumental in helping me develop my teaching skills. Dr. Christine Boake's contribution as a committee member and as a mentor for meeting career goals is sincerely appreciated. Many thanks too to Dr. Gary McCracken for broadening my knowledge of Evolutionary Biology and for serving on my dissertation committee, and to Dr. James Lawler for serving on my SAC and dissertation committees. Richard King (Northern Illinois University) was immensely helpful with statistical and computing issues, as was Mike O'Neil at the U.T. Statistical Consulting Center. Paul Andreadis, Matthew Bealor, and Ruthanne Krause provided many helpful editorial suggestions.

Financial support for this research was provided by The University of Tennessee Alumni Foundation, Science Alliance, and through a SARIF Award. I thank The University of Tennessee, the Department of Psychology, and the faculty and administrators who awarded me the funds.

My colleagues and friends have been an endless source of support, humor, lightheartedness, and education. Mark Waters and Paul Andreadis, my "big brothers" of the Reptile Ethology Lab, were extremely helpful in assisting me with developing research ideas and support all along the way. Matt Lanier, Barbara Manzer, and Julia

Albright were extremely helpful as lab managers, and were good friends to work with day in and day out. I also extend a very warm thank you to all of my undergraduate assistants: Casey Newton, Jayme Tims, Chrisann Lee, Janet Bryant, Toby Chenowith, Amanda Graham, and Amanda McMahon. These individuals were a real pleasure to work with and made normally tedious data entry and collection sessions fun.

A very special thanks to Dr. Jim Gillingham, Director of the Central Michigan University, Beaver Island Biological Station. Dr. Gillingham provided very inexpensive room and board, free vehicle usage, and the use of laboratory space and equipment during my field seasons. His support made a huge portion of my dissertation possible. Thanks to Pedro doAmaral for helping me with computer set-ups while on Beaver Island. Dr. John Rowe was a huge help in the field. Many thanks to John for helping me with capturing garter snakes, identifying stomach contents, seining through marshes and lakes with me, for always providing advice on how to be successful in the field, and for showing me what there is to do in the evenings on Beaver Island. Kenneth Bowen, Casey Elliot, Jennifer Paquette, and many other staff and students at the Biological station were extremely helpful to me while on the island.

Thanks to my parents, James and Ruthanne Krause, and my brother, Matt Krause, for believing in me, listening to me, and accepting my chronic tension and grouchiness these past six years. My friends in the Austin Peay Building kept me safe and sane. Thank you Dr. Dan Corts, Rob Riethmiller, Dennis Plant, Jennifer Siciliani, Warren Roberts, Daniel Knight, Dr. Christopher Heaps, and the rest of you. You all made these years even more enjoyable than my undergraduate ones, and that says a lot.

ABSTRACT

Successful predation is a key component to the survival of snakes. Snakes that encounter periodic fluctuations in prey availability, or that move into novel feeding niches, must be behaviorally and morphologically equipped to adjust to new environmental conditions. Generalists, like the common garter snake (*Thamnophis sirtalis*), thrive in a wide variety of environments. The wide geographic distribution and considerable inter-population variability of *T. sirtalis* highlight their adaptability as a species. While microevolutionary change is known to contribute to the morphological and behavioral diversity of *T. sirtalis*, phenotypic plasticity is prevalent as well. In contrast to prey specialists, *T. sirtalis* feeds on a great diversity of prey species, each of which places different demands on the snakes' sensory and behavioral capacities. In such a generalist, relatively high levels of morphological and behavioral plasticity can be expected. The purpose of my study was to examine the relationship between diet and morphological variation in garter snakes, and how diet influences the ontogeny of chemosensory responses and predatory behavior.

I examined the association between diet and morphological and behavioral differences in *T. sirtalis* inhabiting two sites on Beaver Island in Lake Michigan. Body and head size variation in 457 garter snakes from two ecologically dissimilar habitats were measured over the course of two field seasons (1998-1999). At one of the sites, Miller's marsh, the snakes eat a wide diversity of amphibian species, as well as earthworms. Snakes at the second site, McCafferty farm, consume earthworms almost exclusively. The dietary differences between the two sites are due to differences in prey

availability among sites. Probably owing to dietary differences among sites, adult snakes at Miller's marsh were larger than snakes at McCafferty farm. However, only adult females significantly differed in body size between sites. There was a significant sex by site interaction for body length, suggesting differences in the degrees of sexual dimorphism between the two sites. Controlling for body size, relative head sizes differed among the sites, but this was significant for only one of the four measurements, inter-ocular distance. In addition to morphological measurements, snakes from both sites were tested on their abilities to consume live frog, fish, and worm prey. Overall, adult snakes from both sites did not substantially differ in their abilities to capture, handle and consume these prey items.

I conducted developmental studies of neonates born to mothers from both sites during both years. Females from Miller's marsh had larger litters than females from McCafferty farm; however, the regressions of maternal snout-vent length (SVL) on litter size and neonatal length and mass were low and non-significant (n = 33 litters). I tested for the effects of litter, sex, and site on morphological and behavioral traits. Postpartum morphological analyses revealed significant sex and litter differences in SVL, body weight, and head size. Males had greater SVLs and tail lengths than females, but females were heavier and had larger heads. Site did not influence neonatal SVL or weight. However, neonates from Miller's marsh had significantly longer jaw lengths and inter-ocular distances than neonates from McCafferty farm. Neonates were reared on diets of fish, worms, or both, and growth rates were measured at 80-day intervals until the snakes reached 240 days. Diet had a significant effect on SVL and mass, but did not influence

relative head sizes. Snakes reared on a mixture of fish and worms grew longer and were heavier at 240 days than snakes feeding on single diets. Sex-based differences in head size persisted through 240 days.

I also examined the influence of diet and diet switching on the development of chemosensory responses to prey. Neonatal snakes were divided into diet groups comprising live fish, worms, or both. Tests for chemosensory responses to surface extracts of fish and worms were done prior to feeding experience and at two 80-day intervals following feeding experience. Snakes that had fish in their diet significantly increased their responses to this prey after feeding experience, whereas snakes reared on worms did not reveal a bias toward worm or fish extract. When the diets of the snakes in the fish and worm groups were switched at 160 days, chemosensory responses to fish and worm stimuli were not significantly different for either group when re-tested at 240 days.

Three experiments examined the role of learning and memory in the development of predatory skills. In Experiment I, the snakes used in the growth study were tested for their abilities to approach, capture, handle, and swallow prey at their first feeding, and were twice re-tested at 80 day intervals (11-12 feedings per interval). Diets were then reversed for the groups feeding exclusively on fish or worms, and the same behavioral measures as above were recorded for the first feeding on the new prey, and again after 11-12 feedings. A final trial tested the snakes' retention for consuming the prey comprising their initial diets. Snakes in all three diet-groups decreased their overall latencies to consume prey (e.g., capture, handle, and swallow) after feeding experience. However, snakes feeding initially on worms were slow when consuming fish after diet

switching, whereas snakes that initially fed on fish rapidly consumed worms upon their first feeding. Snakes who had switched to fish decreased their total consumption times after 11-12 feedings. Feeding skills for initial prey were retained following the diet-switching phase.

In Experiment II, the amount of feeding experience prior to diet switching and after diet switching was reduced (6 feedings per group). This was done to assess the effects of shorter durations on each diet. No effect of diet switching or decrement in feeding skills was detected. However, the number of snakes completing the study was small and variation was very high. Individual differences were important contributors to this variation and are described in some detail.

Experiment III was conducted to determine the long-term effects of feeding experience on prey consumption times. Snakes from McCafferty farm and Miller's marsh were tested on their abilities to approach, capture, handle, and swallow frogs, fish, and worms. Although total consumption times for all three prey did not differ among the adult snakes from both sites, there were differences among the four predatory phases measured. Actual feeding experience may be less important for adults than for neonates.

Morphological and behavioral plasticity accounted for much of the variation that I observed. Results from the morphological studies and four behavioral experiments revealed phenotypically plastic responses to varying environments, although important exceptions were found. Behavioral differences due to microevolutionary change were not detected, probably due to either the close proximity of the two sites that I studied, or to larger intra site variability. Plasticity is known to buffer the effects of natural selection

and allows organisms to adjust to environmental variability. The high level of morphological and behavioral plasticity found in garter snakes is a viable explanation of their wide distribution and success as a species.

TABLE OF CONTENTS

CHAPTER PAGE

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

The early experiences that young animals have with prey, or prey related cues, can have a considerable influence on adult predatory behavior. Kuo (1930, 1938) found that kittens with early predatory experience, or that had witnessed predation on rats by conspecifics, were more likely to become rat predators than kittens without these experiences. One primary aim of Kuo's early work was to dispute the concept of instinct as formulated by early ethologists (Kuo, 1967) and his work represents one of the earliest attempts to experimentally investigate the ontogeny of predatory behavior. Subsequent work has examined the diversity of tactics employed by predators in capturing prey, as well as the roles played by sensory, behavioral, and ecological factors in the acquisition of predatory skills (e.g., Caro, 1980; Curio, 1976; Leyhausen, 1973; Polsky, 1975, 1977). Generalist predators consume on a variety of prey types and experience is known to underlie the acquisition of feeding skills. Even precocial species such as snakes rely on postnatal experience for learning how to detect, capture, and consume prey. For predatory generalist species of snakes, high levels of behavioral plasticity facilitates the acquisition of feeding skills, often on novel prey types. The plasticity of predatory behavior in a highly precocial and generalist species, the common garter snake (*Thamnophis sirtalis*), is the topic of my dissertation. My primary objective was to determine how dietary variation affects individual and population differences in morphological and behavioral phenotypes associated with feeding.

Phenotypes vary across spatial and temporal scales, and the amount of variation within a species is often directly proportional to environmental variation, either

seasonally or across its range (Komers, 1997). A specialist strategy may be favored in a constant environment, and a generalist strategy may be favored when environmental variability is high (Gilchrist, 1995). The common garter snake evinces a very level of morphological and behavioral variation (Burghardt & Schwartz, 1999; Gregory & Larsen, 1993). Across its widespread geographic range, *T. sirtalis* exhibits a high degree of phenotypic variation in body size and feeds on a diverse array of species including fishes, worms, leeches, adult and larval amphibians, mammals, and occasionally birds (see Rossman, Ford, & Seigel, 1996). Diet is known to affect growth rates and adult body sizes of snakes (Scudder-Davis & Burghardt, 1987; Queral-Regil & King, 1998) and is one primary contributing factor to snake morphological variation. I examined diet-induced morphological variation in laboratory born neonates and in adult snakes inhabiting two highly different feeding niches. Studies of geographic variation of behavior or morphology often compare populations separated by relatively great distances (see chapters in Foster & Endler, 1999). I examined morphological and behavioral variation in snakes from two sites separated by only 10 km. I reasoned that the close proximity of the two sites minimized the chances that genetic divergence could account for any morphological and behavioral differences observed. Thus, phenotypic plasticity is hypothesized as the likely mechanism accounting for diet-induced behavioral and morphological variation. I also examined the development of several behavioral traits associated with predation, including prey detection and consumption, and chemosensory responses.

The Ontogeny of Behavioral and Morphological Plasticity

Phenotypic plasticity can be defined as "...the ability of a single genotype to produce more than one alternative form of morphology, physiological state, and/or behavior in response to environmental conditions" (Pigliucci, et al., 1996). Definitions of phenotypic plasticity can encompass learned behaviors (Foster, 1999; Pigliucci, in press; Wcislo, 1989). Learning allows animals to rapidly adjust to novel circumstances, and learned behaviors are among the most highly plastic (Komers, 1997). Learning may be a special type of phenotypic plasticity because it facilitates extensive phenotypic modification with the potential of reversibility, often throughout ontogeny. Morphological traits may constrain or facilitate the development and expression of behavioral traits. For example, morphological traits associated with feeding (e.g., skull size and shape, body size) can vary as a function of dietary experience in snakes (Forsman, 1991, 1996a), as well as in many other animals. Robinson and Wilson (1995) report that morphological variation in body, skull, and fin size in Trinidadian guppies (*Poecilia reticulata*) was influenced by body orientation while foraging. Walls, Belanger, and Blaustein (1993) found diet-induced changes in trophic morphology of larval salamanders (*Ambystoma macrodactylum*). Larval salamanders raised on a varied diet that included relatively large prey items as compared to groups reared on more size-restricted diets, showed significant increases of various head measurements in relation to body size. Losos et al. (2000) found that Anolis lizards (*Anolis sagrei*) inhabiting niches with broad surfaces (perches) had long hindlimbs, whereas those inhabiting narrow substrates had relatively short hindlimbs. In both cases, limb length facilitated efficient movement. Hatchling lizards reared in environments with broad substrates developed

longer hindlimbs than those reared on narrow substrates. Thus, similar to behavior, morphological traits such as skeletal size and shape undergo changes as a function of environmental input (Lanyon & Rubin, 1985), particularly during growth phases of development.

Morphological Plasticity in Snakes

Variations in characteristics of snakes such as body size, length, and head shape are often attributable to food quantity (Forsman, 1996a,b), diet (Lyman-Henley & Burghardt, 1995; Scudder-Davis & Burghardt, 1987), and prey size (Queral-Regil & King, 1998; Shine, 1991). However, sexual size dimorphism is also widespread in snakes and sex can be an important determinant of body and head sizes (Shine, 1990, 1994; Shine & Crews, 1988). Also, studies of geographic variation and dietary effects on snake morphology have consistently demonstrated correlated head size variation in a few species of snakes, including adders (Forsman, 1996a), garter snakes (Grudzien et al., 1992), and water snakes (Queral-Regil & King, 1998).

Forsman (1991) found phenotypic variation in head length among mainland and island populations of European adders (*Vipera berus*). Adders inhabiting islands with large voles (*Microtus agrestis*) had longer heads (relative to body size) than adders living on islands with smaller voles. The differences among the islands could indicate that microevolutionary changes have altered the relative head sizes of these snakes or that inter-island variation is due to plastic responses to dietary differences. In a laboratory study, Forsman (1996a) reported significantly greater body sizes of snakes fed twice weekly compared to snakes fed once weekly on the same species of prey. Therefore, local variation due to phenotypic plasticity may be the simplest explanation for size differences

found in natural populations of adders. Similarly, Madsen and Shine (1993) report phenotypic plasticity of body size in European grass snakes (*Natrix natrix*) among mainland and island populations. Smaller body sizes were found among snakes inhabiting an island with relatively small prey items. Larger snakes were able to consume larger prey, and consumed prey more rapidly, than did smaller snakes (Shine, 1991). Differences in body and head size were explained as adaptive responses to food availability, with variation attributed to phenotypic plasticity.

The studies on morphological variation summarized above (e.g., Forsman, 1996a; Forsman & Lindell, 1993; Madsen & Shine, 1993) were comparisons of snake populations separated by considerable geographic distances (e.g., island versus mainland). However, morphological and behavioral variation can occur between sites that are separated by very small distances. Recently, Brown and Weatherhead (1999) reported geographic variation of fat reserves in male water snakes (*Nerodia sipedon*) separated by only 1km. Thus, population or site variation can occur at very small scales and when genetic differences are minimal or non-existent. I examined head and body size variation in garter snakes inhabiting two sites on Beaver Island in Lake Michigan (Charlevoix County, MI), Miller's marsh, and McCafferty farm, which are separated by a comparatively small distance (10km). Beaver Island is located 18 miles from the lower Michigan peninsula, and was created after the last glacial period, roughly 9000 years ago. The island measures a maximum of 21 km long and 10.5 km wide, and is the largest of several islands in an archipelago that extends north to south.

Much of the work done on the Beaver Island *T. sirtalis* has taken place at or near McCafferty farm (Burghardt, unpublished data; Gillingham, Rowe, & Weins, 1990),

where the snakes primarily feed on earthworms. Miller's marsh hosts a much wider variety of prey items, including several species of amphibians, as well as earthworms. Information on the potential prey species available on Beaver Island (Table 1.1) comes from Gillingham (1988), Dickinson (1979), and from stomach content analyses of *Thamnophis sauritus* (Gillingham, unpubl. data) at Miller's marsh. Due to their amphibian-rich diet, the snakes from Miller's marsh should have greater body lengths, weights, and head sizes than the worm-feeding snakes inhabiting McCafferty farm. The greater body sizes of amphibians and the higher level of nutrients they provide in comparison to earthworms should result in greater body lengths and weights. Furthermore, the greater body sizes of amphibians, the presence of hard body parts, and the greater challenge they present in handling and swallowing (compared to worms) should result in greater head sizes in the snakes from Miller's marsh. Sex differences in morphology are also expected. Sexual head and body size dimorphism in snakes is widespread, and the degree to which the sexes vary depends on a variety of phylogenetic and ecological factors (see Shine, 1993 for review). In *T. sirtalis*, females are generally longer and heavier than males and have greater relative head sizes (see Crews et al., 1985; King et al., 1999). Based on previous findings with *T. sirtalis*, I predicted the same pattern for my study.

In addition, I predicted neonatal site and sex differences in morphological traits. Ford and Seigel (1989) report significantly greater clutch masses in garter snakes (*Thamnophis marcianus*) reared on high-energy diets (30% of snake body mass) compared to snakes reared on low-energy diets (10% of snake body mass). Thus, garter snakes born to females collected at Miller's marsh should have greater mean body

Table 1.1: Potential prey species (including adults and larvae) of *T. sirtalis* inhabiting Beaver Island.

Frogs and toads	Salamanders and newts
*American toad (*Bufo americanus*)	*Red-spotted newt (*Notophthalmus viridescens*)
*Spring Peeper (*Hyla crucifer*)	*Red-backed salamander (*Plethodon cinereus*)
*Gray tree frog (*Hyla versicolor*)	*Spotted salamander (*Ambystoma maculatum*)
*Wood frog (*Rana sylvatica*)	*Blue-spotted salamander (*Ambystoma laterale*)
Green Frog (*Rana clamitans*)	Annelids
Bullfrog (*Rana catesbeiana*)	Earthworms
Birds	Mammals
Red-wing blackbird (*Agelaius phoeniceus*)	Red-backed vole (*Clethrionomys gapperi*)
Virginia rail (*Rallus limicola*)	Snowshoe hare (*Lepus americanus*)
	Shrew (*Blarina brevicauda*)

Note: At least one instance of predation was recorded for each of these species, and were found in adult snakes.
* These are species that have also been found in the stomachs of *Thamnophis sauritus* at Miller's marsh (Rowe & Gillingham, in prep.).

weights and/or body lengths than snakes born to mothers from McCafferty farm. Head size differences at birth are not expected to occur among sites, because variation in head size is often induced by diet (e.g., Queral-Regil & King, 1998). Based on the findings of King et al. (1999), neonatal females from both sites are expected to have greater body weights and head sizes than males. Males are expected to have greater body and tail lengths. The following hypotheses are tested in Chapter 2, "Growth and Morphology":

1) Adult garter snakes of both sexes from Miller's marsh will have greater SVLs, body weights, and RHSs than snakes from McCafferty farm.
2) Females from both sites will be larger and will have greater RHSs than males.
3) Neonatal garter snakes born to mothers collected at Miller's marsh will be longer, heavier, or both.
4) Neonatal garter snakes born to mothers from both sites will not differ in relative head size.
5) Garter snakes reared on fish diets, or a mixed diet consisting of fish and worms, will grow more rapidly than snakes reared on worms only.

Ontogeny and Plasticity of Garter Snake Chemosensory Responses

The dominant chemosensory channel for most snake species is the vomerolfactory system (Cooper & Burghardt, 1990), in which the tongue gathers chemical cues and transports them to sensory receptors on the vomeronasal organ, which relay messages to the central nervous system (Halpern, 1992). This complex system allows snakes to locate prey, predators, and conspecifics (see Burghardt, 1970a; Ford & Burghardt, 1993; Halpern, 1987 for reviews). Cooper and Burghardt (1990) and Cooper (1998) discuss methods of measuring and analyzing data on reptile chemosensory responses. The 'swab

test', an indicator of chemosensory investigation and discrimination, involves soaking a cotton-tipped swab in prey extract, or rubbing it along the surface of live or dead prey, and presenting the swab tips to subjects. Tongue-flick rates and latencies to attack swabs are measured and the data are converted into tongue-flick/attack scores (Cooper & Burghardt, 1990). The swab test minimizes or eliminates visual and tactile cues, thereby restricting stimulation to the vomeronasal system. The term "vomodor" (Burghardt & Cooper, 1990; Cooper, 1998) refers to chemical cues exposed to snakes with the methods described above.

Burghardt (1966, 1967, 1969) found that, prior to feeding experience, neonatal snakes of various species will tongue flick toward and attack swabs dipped in aqueous solutions of different prey. Since these initial studies, this method has been implemented to address a variety of developmental, ecological, and evolutionary questions (Arnold, 1992; Burghardt, 1993; Burghardt, Layne, & Konigsberg, 2000; Cooper, 1998). Chemically based preferences by snakes can be assayed with this method and response profiles often correspond with the natural diets of the snakes tested (Arnold, 1981a; Burghardt, 1967, 1970b; Burghardt & Schwartz, 1999; Mushinsky & Lotz, 1980).

Although the snake vomerolfactory system is functional at birth and preferences for certain prey types may be present at birth, response levels can be modified during maturation and with different types of feeding experience (Arnold, 1978; Burghardt, 1993; Burghardt, et al, 2000; Burghardt, Wilcoxon, & Czaplicki, 1973; Fuchs & Burghardt, 1971; Gove & Burghardt, 1975; Mushinsky & Lotz, 1980). For example, adult *T. sirtalis* collected from the Beaver archipelago in northern Lake Michigan responded more strongly to amphibian and fish surface extracts than neonates born to

mothers in the same area, suggesting a long term effect of diet on chemosensory responses (Greenwell, Hall, & Sexton, 1984). Even exposure to prey chemicals alone prior to feeding experience is known to affect chemosensory responses to prey (Burghardt, 1992).

Diet effects on chemosensory responses have been detected in very young snakes (neonatal to 159 days: Arnold, 1978; Fuchs & Burghardt, 1971; Lyman-Henley & Burghardt, 1995). Arnold (1978) found that *T. sirtalis* reared on fish show a response bias toward fish vomodors in comparison to snakes fed worm or amphibian diets. Fuchs and Burghardt (1971) found that neonatal garter snakes respond more strongly toward familiar prey (fish or worms) through their first 42 days of age. *Thamnophis butleri*, an earthworm specialist, that were reared on fish through their first 159 days responded more strongly to fish extract than snakes reared on worms (Lyman-Henley & Burghardt, 1995). Of additional interest is whether chemical prey preferences are consistent through longer developmental time periods, and whether switching to a new diet affects chemosensory responses at a later age than previously reported (Fuchs & Burghardt, 1971).

The chemical prey preferences of young garter snakes may change with feeding experience because effective detection of previously eaten prey may increase foraging success. This would be especially important in very young snakes that, due to their restricted body sizes, are limited in the type of prey they can consume. I examined relatively long-term effects of diet and diet switching on chemosensory responses in *T. sirtalis*. In my experiment, snakes were reared on diets of fish, worms, or both. Tests for chemosensory responses to these stimuli were done shortly after birth (prior to feeding

experience) and at 80 and 160 days. At 160 days, the diets of the fish and worm-fed snakes were reversed and responses to surface extracts of both prey were re-tested at 240 days. The experiment reported in Chapter 3, "Experiential Modification of Chemically Mediated Responses", tested the following hypotheses:

1) Neonatal garter snakes will not show any response biases toward fish or worm extracts prior to feeding experience.
2) Following feeding experience, a response bias to fish extract will occur among snakes at 160 days that are fed exclusively on fish. The snakes reared exclusively on worms are predicted to show no preference for either stimulus at 160 days. The snakes feeding on the mixed diet will show a greater chemical preference for fish than for worms.
3) Snakes reared on worms through 160 days will show a response bias toward fish after their diets are switched to fish. The snakes reared on fish initially will not show an increased preference for worm extract after their diets are switched to worms, and their response levels to fish extract will decrease after the diet switching phase. The snakes in the mixed-diet group will still show a greater preference for fish extract over worm extract.

Ontogeny and Plasticity of Garter Snake Feeding

Detecting, subduing, and consuming prey are vital aspects of behavior in animals that forage actively, and feeding experience often plays an important role in the development of foraging skills. The literature on the role of ontogeny in the acquisition of foraging skills encompasses a wide variety of species, including invertebrates (Kause,

Haukioja, & Hanhimaki, 1999; Serra, Chellazzi, & Castilla, 1997), fish (Croy & Hughes, 1991a,b; Day & McPhail, 1996), birds (Yoerg, 1994), and squamate reptiles (Burghardt & Krause, 1999; Halloy & Burghardt, 1990; Mori, 1996). Species that feed on multiple prey types may have to learn several different feeding strategies in order to consume prey. Furthermore, predators may also weigh the costs and benefits of feeding on different available prey. The profitability of a prey species can be measured by the energy yield it provides, and by the amount of time it takes a predator to detect, capture, and consume it relative to other prey species (Hughes, 1979; Stephens & Krebs, 1986). For example, shorecrabs (*Carcinus maenas*) switch to new prey species if net energy yields increase and if handling times for the new prey are not significantly greater than handling times for the previous prey (Cunningham & Hughes, 1984). Reducing the amount of time spent foraging may also reduce predation risk (Milinski & Heller, 1978), and one way of accomplishing this is to learn how to better detect, capture, and consume prey. Thus, animals that evince high levels of plasticity in their foraging behavioral repertoires can acquire feeding skills on a variety of prey species.

For many species, feeding experience underlies the development of successful foraging. However, the type of experience places limits on the degree to which feeding skills (e.g., reduced time spent foraging or net energy spent foraging) can improve. For example, both successive switching between dissimilar prey items, and simultaneous, mixed experience with multiple prey items, can impede the development of efficient foraging behavior in shorecrabs, *Carcinus maenas* (Cunningham & Hughes, 1984). Burghardt & Krause (1999) found that newborn garter snakes feeding on a mixed diet (fish and worms) did not consume worms as quickly as snakes fed exclusively on worms.

Memory is an important factor to consider in determining the effects of diet on the development of foraging skills. For sticklebacks (*Spinachia spinachia* L.), the retention of foraging skills can be impeded when diets are variable or if a substantial time interval passes between feedings (Croy & Hughes, 1991a).

The morphology and behavior of prey can have a considerable influence on the development of predatory skills. Specifically, the type of prey consumed may influence the degree to which foraging times can be reduced following feeding experience. For example, Croy and Hughes (1991a) reared fifteen-spined stickleback (*Spinachia spinacha* L.) on pelagic brine shrimp (*Artemia* sp.), benthic amphipods (*Gammarus locusta*), or a mixture of both, and assessed the development of feeding skills on all three diet groups. Feeding skills on *Artemia*, which is very slow moving and soft-bodied, were acquired more quickly than skills on *Gammarus*, which is fast moving and covered with a hard cuticle. Feeding on a mixed diet impeded the development of feeding skills relative to the groups reared on single item diets. However, it is possible that effective foraging on a new prey type after feeding experience on a different prey type may be facilitated if the organism's initial diet consisted of a prey type that was relatively difficult to consume.

Efficiently detecting, capturing, and consuming prey may minimize predation risks for garter snakes that forage in open fields, along water banks, and under water, where they themselves may be vulnerable to predators. The rapid acquisition of feeding skills by *T. sirtalis* would aid in reducing the costs of not specializing on a limited number of prey species. Behavioral plasticity would facilitate the acquisition of feeding on both novel and species-typical prey. For example, *T. sirtalis* in most populations feed on earthworms and amphibians, but will feed on fish opportunistically in the field and

readily in captivity (Arnold, 1992; Carpenter, 1952; Dix, 1968; Gregory & Nelson, 1991). However, neonatal *T. sirtalis* are not very adept at handling live fish in comparison to *Thamnophis melanogaster*, an aquatic prey specialist (Halloy & Burghardt, 1990). With feeding experience, *T. sirtalis* is capable of consuming fish about as proficiently as *T. melanogaster*, thus benefiting from behavioral plasticity.

The feeding behavior in *T. sirtalis* shows a considerable level of plasticity in the first few months of life. Burghardt and Krause (1999) tested three groups of neonatal *T. sirtalis* on their abilities to feed on fish, worms, or a mixed diet. Initially, all prey items took equally long to consume. However, after 11 to 12 feedings on their respective diets, both fish and worm consumption times decreased significantly. Also, the subcomponents of feeding were differentially affected by feeding experience. Fish and worm detection, as measured by prey approach times, decreased significantly after feeding experience with pure diets but not for snakes reared on mixed diets. Feeding on a mixed diet also appeared to interfere with the development of abilities to approach, capture, handle, and swallow worms.

The findings reported in Burghardt and Krause (1999) lead to further questions about the ontogeny and plasticity of garter snake foraging behavior. The first question concerns the age at which young snakes reach asymptotic levels of prey consumption efficiency. Second, the effects of food switching need to be further addressed. It appears that feeding on fish, a prey item that is relatively difficult to consume, may facilitate switching to worms, which are easier to handle. Conversely, feeding on worms may interfere with switching to fish (Yeager, Burghardt, & Lyman-Henley, 1996). Third, garter snakes may experience periodic fluctuations in prey abundance, which can result in

the absence of certain prey types for extended periods of time. Thus, the retention of feeding skills by garter snakes needs to be assessed. Fourth, the relationships between feeding experience, foraging efficiency, and prey type need to be examined in snakes from natural populations. The following hypotheses were tested in Chapter 4, "The Ontogeny of Feeding Skills":

Experiment I.

1) Latencies to approach and consume prey will decrease significantly after 11 to12 feedings for snakes reared on fish, worms, and a mixed diet. Feeding skills will asymptote by this point and an additional 11to12 feedings will not affect times to approach and consume prey (11 to 12 feedings were offered so that results could be compared with Burghardt and Krause (1999) and because the effects of physical maturation are minimized by re-testing the snakes after a relatively small number of feedings).
2) Feeding on pure diets of fish or worms will result in greater decreases in approach and consumption times in comparison to snakes reared on the mixed diet.
3) Snakes reared on worms for their first 22 to24 feedings will not feed efficiently upon fish when their diet is switched (interference effect), and snakes reared on fish their for their first 22 to24 feedings will feed efficiently on worms when their diet is switched (facilitation effect).
4) Skill retention for consuming fish and worms will be evident after diets are switched back to the original prey item.

Experiment II.

1) Prey approach and consumption times will decrease after only six feedings (half of the number used in Experiment I) for snakes reared on fish and worm diets.
2) Interference and facilitation effects will occur when diets are reversed for the worm and fish fed snakes respectively.
3) Skill retention for consuming fish and worms will be evident after diets are switched back to the original prey item.

Experiment III.

1) Adult snakes from Miller's marsh and McCafferty farm will detect and consume worms at the same rate.
2) Adult snakes from Miller's marsh will approach and consume frogs and fish more rapidly than snakes from McCafferty farm.

Morphological and behavioral adaptations to different feeding niches are well documented for many species of snakes (Drummond, 1983; Halloy & Burghardt, 1990; Waters, 2000). The high levels of plasticity found in generalist species such as *T. sirtalis* provide plausible explanations for their success in invading new food niches. The dietary differences in the snakes at the two sites studied here form the basis for several of the hypotheses tested in this dissertation. Differences in diet, morphological traits, and behavior among sites and sexes were examined using data gathered over the course of two field seasons. The laboratory studies of chemoreception and prey feeding skills assessed the effects of diet on the development of garter snake predatory behavior.

CHAPTER 2

GROWTH AND MORPHOLOGY

Introduction

Thamnophis sirtalis from several mainland and island populations of the Beaver Archipelago differ in relative head sizes, and this appears to correspond with differences in available diets (Grudzien et al., 1992). Garter snakes on island populations of the Beaver Archipelago are known to consume birds whereas those on the mainland do not. Thus, the diets of the snakes in the island populations may be more diverse than the diets of the snakes in the mainland populations (Grudzien et al., 1992). The first part of this chapter examines morphological variation associated with diet differences in snakes from Miller's marsh and McCafferty farm. Several authors have demonstrated that diet affects the head and body sizes of snakes (Forsman, 1996a; Madsen & Shine, 1993; Queral-Regil & King, 1998, see Chapter 1), and morphological plasticity is often evident. For example, *Thamnophis sirtalis* and *T. radix* feeding on fish grew at a faster rate than snakes feeding on worms (Scudder-Davis & Burghardt, 1987). This was likely due to the higher levels of calcium and phosphorous available in the fish diets, as snakes feeding on worms supplemented with these minerals grew even more rapidly than snakes feeding exclusively on fish (Burghardt, 1990; Lyman-Henley & Burghardt, 1995). Water snakes (*Nerodia sipedon*) feeding on large fish had greater body and head sizes than snakes that ate an equal number of smaller fish (Queral-Regil & King, 1998). I tested whether dietary differences are associated with head and body size differences between the two sites. Furthermore, variables such as sex also influence body and head size differences in snakes. Many species of snakes show either male or female biased sexual size

dimorphism (see Rivas & Burghardt, submitted; Shine, 1993 for reviews). The adults of many species of natricine snakes, including *T. sirtalis,* show female biased sexual size dimorphism (King et al., 1999; Shine, 1993, 1994). In this chapter I also tested for sex differences in body and head sizes of wild-caught snakes.

The majority of studies examining sexual size dimorphism in snake body and head sizes have used adult snakes. Several possible explanations for adult head size dimorphism in snakes have been proposed. Crews et al. (1985) and Shine and Crews (1988) found that male *T. sirtalis* have smaller heads than females because the higher androgen levels in males inhibit growth. This finding raises the possibility that sexual selection accounts for sexual size dimorphism in garter snake head sizes. However, sexual selection is an unlikely explanation for head size dimorphism, as male garter snakes are not noticeably aggressive toward one another during their attempts to mate with females. If female mate choice exists among garter snakes, it appears to have little to do with male head size. An alternative hypothesis is that sexually dimorphic snake species utilize different food niches. Some support for this hypothesis comes from Shine (1986). Alternatively, sex differences in garter snake head size may be an incidental effect of androgen levels and there may be no adaptive explanation. This is unlikely as males of many species of snakes have larger heads than females, and there are populations of *T. sirtalis* where head sizes do not differ among sexes (Shine & Crews, 1988). Recently, King et al. (1999) reported sexual size dimorphism of body and head sizes in neonatal *T. sirtalis* from Ohio (Ottawa County). In this chapter, I tested whether sexual size dimorphism of body and head sizes occurs in neonatal *T. sirtalis* born to mothers collected at Beaver Island.

At birth, body lengths of *Thamnophis elegans* differ among litters, and relative size differences among individuals at birth persist through the first year of growth (Gregory & Prelypchan, 1994). Thus, in this chapter I also tested for litter and site variation in neonatal body weight, SVL, and relative head size. I also reared laboratory born snakes on diets of fish, worms, or both, to determine the degree to which diet and sex influence increases in body weight, SVL, and head size during the first 240 days of age. Due to high mortality through the first 240 days of age, litter effects on growth could not be tested.

Below, I have reported head and body size data on wild-caught snakes from the two sites, and tested for sex, reproductive condition, and site effects on morphological variation. I then examined sex, site, and litter effects on neonatal morphology using snakes born in captivity to females from both sites. Finally, I tested sex, site, litter, and diet effects on growth in neonates reared on different diets. The specific hypotheses that I tested are listed in Chapter 1.

Method: Field Data

Subjects and maintenance

Field data were gathered at Miller's marsh and McCafferty farm between May and July in 1998 and 1999. I captured snakes by hand and brought them to the Central Michigan University, Beaver Island Biological Station for measurements. I captured and measured a total of 457 snakes during both field seasons. Due to their greater abundance and/or ease of capture, a somewhat larger sample was obtained from McCafferty farm (n=246; 68 males, 178 females) than from Miller's marsh (n=211; 68 males, 143 females). Snakes at Miller's marsh were captured around the perimeter of the marsh and

along adjacent roads. Grass and bracken fern covered berms alongside the unpaved roads created highly suitable garter snake habitat. Snakes were captured at Miller's marsh at least 6 days/week and 3 days/week at McCafferty farm. McCafferty farm consists of a privately owned, 2 ha grass field with scattered boards that the snakes use as refuge and to thermoregulate. Nearly all snakes at McCafferty farm were captured from beneath these boards. At the Biological Station, snakes were group-housed by site. Temperature was kept at 20-25° C and the snakes were housed near windows to keep them as close to their natural light-dark cycles as possible.

Procedure

The following morphological measurements were taken on the snakes captured during the 1998 field season (n = 289): Mass, snout-vent-length (SVL), tail length (TL), head length (HL, distance between snout and posterior margin of the parietal scales), jaw length (JL, distance between the snout and the posterior margin of the posterior-most upper labial scale on the left side of the head), head width (HW, widest anterior point to posterior margin of parietal scales), and inter-ocular distance (IOD, distance between junction point of supraocular scale, parietal scale, and eyes). Body mass was measured in 0.01g with a digital scale. SVL and TL were measured in mm with a meter stick and head measurements (in 0.01mm) were made with a hand-held digital caliper. Sex was determined by cloacal probing. Figures showing the location of each head scale can be found in Conant and Collins (1991). Based on findings in 1998, HL, but not JL, HW, and IOD, was measured on snakes captured during the 1999 field season (n = 168) so more effort could be devoted to behavioral testing.

The repeatability of measurements on SVL, HL, JL, HW, and IOD was assessed on fifteen adult snakes captured in 1998 at McCafferty farm following the procedure of Forsman (1994). All fifteen snakes were scale- clipped and placed into an aquarium. The first measurements were then taken, and each snake was placed into a second aquarium. After all fifteen snakes were first measured, the measurements were repeated twice using the same procedure, for a total of three measurements on all variables for each snake. For each repetition, there was no reference to earlier measurements. All repeatability values were calculated using the intra-class correlation coefficient and were extremely high (0.96 - 0.99, Table 2.1).

Dietary differences at the two sites were examined by non-destructive analysis of stomach contents from all snakes captured during the 1998 and 1999 field seasons. The presence of stomach contents was determined by palpating each snake's stomach. If ingested prey were detected, stomach contents were removed by gently pushing the prey forward through the stomach and gullet. This method of stomach content removal, described by Fitch (1987), does not cause injury. Stomach contents were identified, weighed, and then frozen. Snakes were then scale clipped for identification purposes and released at their capture site, typically within 24 hours (see Chapter 4, Experiment III, for some exceptions).

Statistical analyses

Sex and site variation in SVL was tested using ANOVA. Body masses were compared among sexes and sites using a factorial ANOVA, with SVL as a covariate.

Table 2.1: Repeatability of measurements for snout-vent length (SVL), head length (HL), jaw length (JL), head width (HW), and inter-ocular distance (IOD) for fifteen wild-caught garter snakes.

	SVL	HL	JL	HW	IOD
R	0.99	0.99	0.99	0.97	0.96
F-value	3210.4	592.7	1254.4	98.0	83.2
p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Simple linear regressions were performed to compare the relationships between SVLs and masses for both sexes and sites. All multivariate analyses of head size were performed on the 289 snakes captured in 1998. Phenotypic correlations between HL, JL, HW, and IOD were calculated separately for both sexes and sites. The calculation of these correlations is complicated by the fact that HL, JL, HW, and IOD increase with increasing SVL. Partial correlations are the appropriate statistical tests to control for this, using SVL as the control variable (King, 1997). An ANCOVA was used to test for sex and site differences in head dimensions, with these two variables treated as fixed factors, and SVL treated as a covariate. Wilk's Lambda (λ) was used to test for multivariate effects of the following factors: SVL, site, sex, and the sex by site interaction. I used univariate F-statistics to test for the effects of each of these factors on HL, JL, HW, and IOD. Data were natural log transformed to normalize the data and to linearize variate-covariate relationships.

Results: Field Data

Due to a relatively small number of snakes with prey (3.9% of all snakes captured), stomach contents were examined in descriptive fashion. Only one stomach content sample, consisting of earthworms, was taken at McCafferty farm (Table 2.2). The weather was dry during both field seasons, possibly making earthworms less available. Worms are also digested more rapidly than vertebrate species (Scudder-Davis & Burghardt, 1987). Additional data on stomach content analyses of snakes from McCafferty farm (Gillingham & Burghardt, unpubl.) have revealed only earthworms. Several species of amphibians were found in the snakes at Miller's marsh, as well as earthworms, a nestling bird, and a shrew (Table 2.2).

Table 2.2: Stomach contents from female (f) and male (m) garter snakes captured with identifiable prey from each site during the 1998-1999 field seasons.

Prey species	Miller's marsh (#stomachs)	McCafferty farm (#stomachs)
Red-backed salamander (*Plethodon cinereus*)	1 (f)	0
Green Frog (*Rana clamitans*)	1 (f)	0
Gray tree frog (*Hyla versicolor*)	6 (5f, 1m)*	0
Spring Peeper (*Hyla crucifer*)	1 (f)	0
American toad (*Bufo americanus*)	1 (f)	0
Spotted salamander (*Ambystoma maculatum*)	2 (1f, 1m)*	0
Shrew (*Blarina brevicauda*)	1 (m)	
Bird (nestling)	1 (f)	0
Earthworms	3 (2f, 1m)	1 (f)

* One female had two *Hyla versicolor* in her stomach, and three *Ambystoma maculatum* were found in another female's stomach.

Site and sex differences in body size

Descriptive results for all morphological measurements taken on all snakes captured at both sites are shown in Table 2.3A. Comparisons of SVL and mass between the two sites and sexes were restricted to adult snakes (Table 2.3B). Female and male adult sizes were estimated using size-frequency histograms (Figure 2.1A-D) from SVL data collected at both sites, and compared to previously published estimates of male and female adult SVL in *T. sirtalis*. Based on the data presented in Figure 2.1A-B, and King (1989), the minimum adult male SVL was estimated at 350.0 mm. A total of 40 yearling and juvenile males were thus omitted from the analysis (Miller's marsh, n = 16, M mass ± SE = 10.2 g ± 0.95, M SVL ± SE = 276.9 mm ± 10.1; McCafferty farm, n = 24, M mass ± SE = 9.2 g ± 0.96, M SVL ± SE = 256.2 mm ± 8.6). The smallest female from McCafferty farm in which embryos could be detected was 392.0 mm, and the smallest female with embryos from Miller's marsh was 422.0 mm. Based on this and the data in Figure 2.1C-D, minimum female adult body size was rounded to 400.0 mm (the next smallest female with embryos at McCafferty farm was 420.0 mm). A total of 72 yearling and juvenile females was omitted (Miller's marsh, n = 29, M mass ± SE = 11.9 g ± 1.19, M SVL ± SE = 286.4 mm ± 11.52; McCafferty farm, n = 43, M mass ± SE = 15.3 g ± 1.29, M SVL ± SE = 300.5 mm ± 9.5). Yearling and juvenile snakes of both sexes were more commonly captured at McCafferty farm, which may be due to their relative ease of capture compared with smaller snakes at Miller's marsh, or because younger snakes are less common at Miller's marsh.

Table 2.3: Mean (± 1SE) mass (g), snout-vent length (SVL), tail length (TL), head length (HL), jaw length (JL), head width (HW), and inter-ocular distance (IOD) (all in mm) for all male and female garter snakes caught at both sites (A), and values for adult snakes only (B).

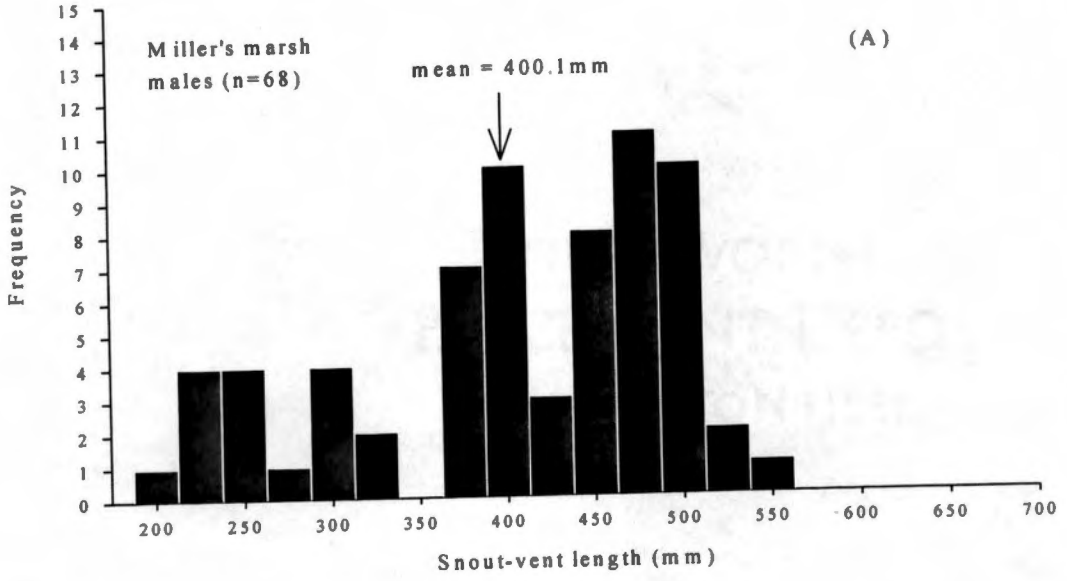
(A)		Mass (0.1g)	SVL (1.0mm)	TL (0.01mm)	HL (0.01mm)	JL (0.01mm)	HW (0.01mm)	IOD (0.01mm)
MM	Males	27.9(1.67) n=68	400.1(10.0) n=68	116.5(3.95) n=68	14.76(0.25) n=68	15.02(0.43) n=32	6.62(0.15) n=32	5.64(0.13) n=32
	Females	67.2(3.20) n=143	490.1(9.74) n=143	128.9(2.78) n=140*	17.27(0.26) n=143	19.57(0.41) n=97	7.94(0.13) n=97	6.64(0.10) n=97
	Totals	54.4(2.56) n=211	461.1(7.89) n=211	124.9(2.31) n=206	16.46(0.21) n=211	18.44(0.36) n=129	7.61(0.11) n=129	6.39(0.09) n=129
MF	Males	27.4(2.03) n=68	373.0(11.87) n=68	113.9(4.19) n=68	14.25(0.32) n=68	14.07(0.43) n=43	6.34(0.15) n=43	5.43(0.14) n=43
	Females	50.9(1.92) n=178	448(7.35) n=178	119.4(2.45) n=176*	16.37(0.20) n=178	17.83(0.30) n=124	7.45(0.01) n=124	6.31(0.08) n=124
	Totals	44.37(1.64) n=246	427.7(6.60) n=246	117.9(2.12) n=244*	15.79(0.18) n=246	16.86(0.28) n=167	7.17(0.09) n=167	6.09(0.08) n=167

Table 2.3 (continued)

(B)		Mass (0.1g)	SVL (1.0mm)	TL (0.01mm)	HL (0.01mm)	JL (0.01mm)	HW (0.01mm)	IOD (0.01mm)
MM	Males	33.4(1.49) n=52	438.0(6.58) n=52	127.3(4.04) n=50*	15.68(0.19) n=52	16.53(0.33) n=20	7.16(0.11) n=20	6.05(0.13) n=20
	Females	81.0(2.73) n=114	541.9(4.89) n=114	141.9(2.06) n=111*	18.55(0.16) n=114	21.19(0.23) n=79	8.42(0.09) n=79	7.05(0.07) n=79
	Totals	66.1(2.58) n=166	509.4(5.43) n=166	137.4(1.96) n=161*	17.65(0.16) n=166	20.25(0.27) n=99	8.17(0.09) n=99	6.85(0.07) n=99
MF	Males	36.6(1.86) n=45	432.8(8.10) n=45	133.3(3.62) n=45	15.80(0.26) n=45	16.60(0.44) n=20	7.18(0.16) n=20	6.27(0.14) n=20
	Females	62.2(1.52) n=135	495(4.03) n=135	129.3(1.85) n=133*	17.62(0.12) n=89	19.68(0.16) n=89	8.02(0.06) n=89	6.80(0.05) n=89
	Totals	55.8(1.48) n=180	480.0(4.16) n=180	130.3(1.65) n=178*	17.16(0.12) n=180	19.11(0.19) n=109	7.87(0.07) n=109	6.70(0.05) n=109

Note: MM=Miller's marsh, MF=McCafferty farm. 1998 and 1999 data are pooled. JL, HW, and IOD were not measured in 1999 which, explains the unequal sample sizes. Adult sizes (Table B) were estimated at 350.0 mm SVL for males and 400.0 mm SVL for females.

*some snakes values were omitted because of tail injury.



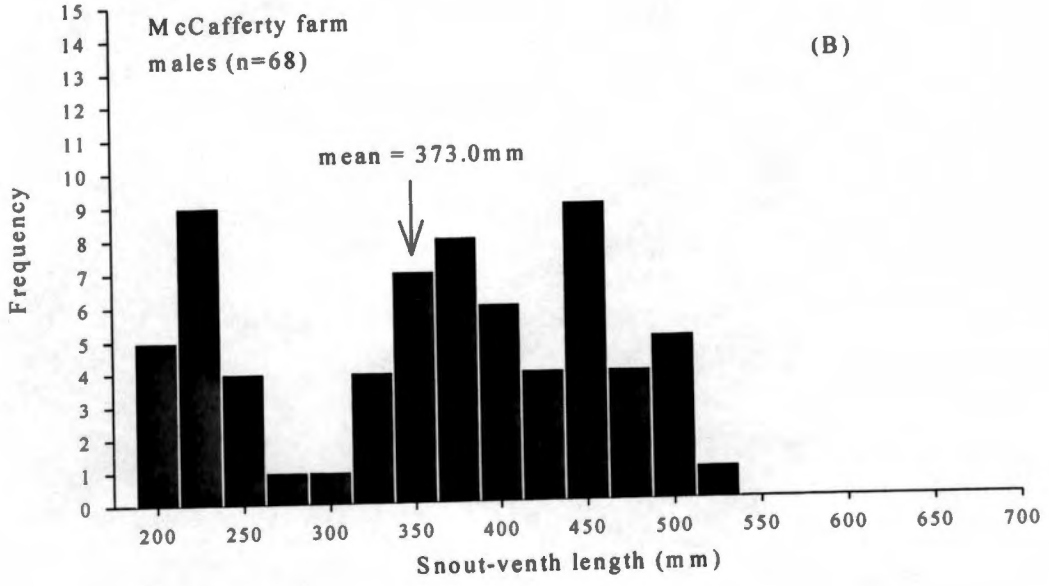
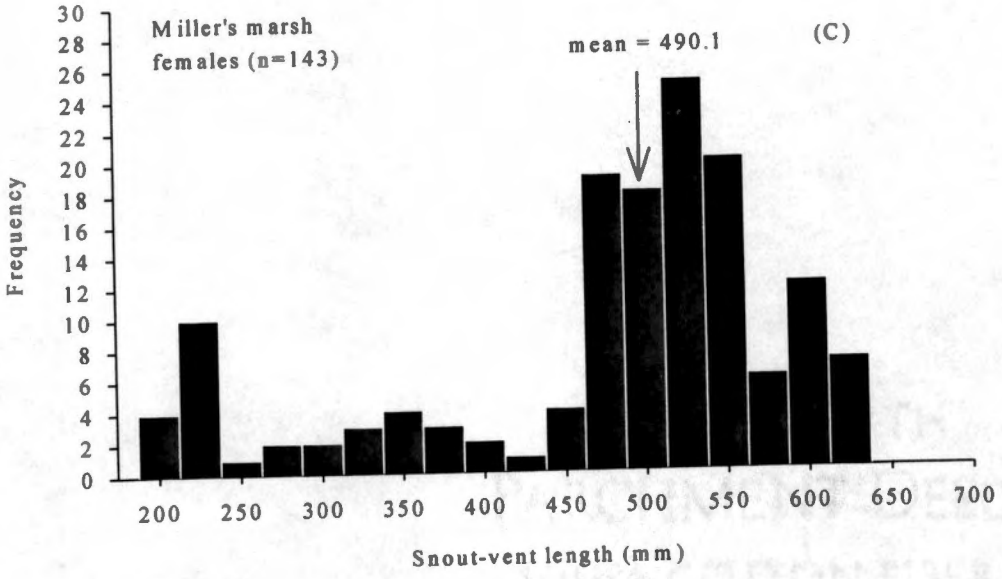


Figure 2.1: Size frequency histograms (SVL) for males from Miller's marsh (A) and McCafferty farm (B) and females from Miller's marsh (C) and McCafferty farm (D).



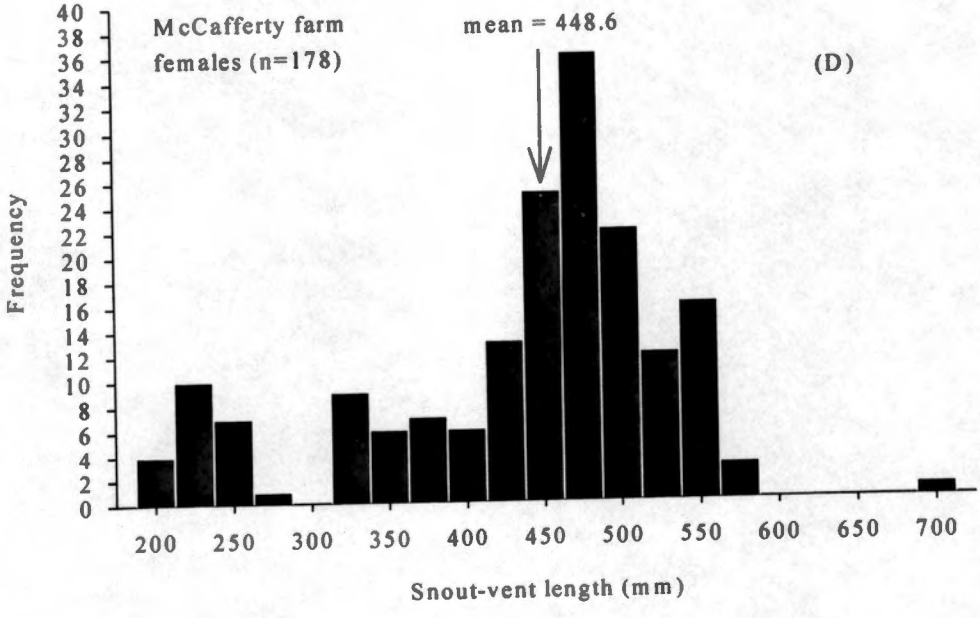


Figure 2.1 (continued)

Descriptive statistics of all morphological traits measured for adult males and females from both sites are reported in Table 2.2B. Sex and site effects on adult SVL were tested using a univariate ANOVA. Site had a significant effect on SVL, with snakes at Miller's marsh having greater SVLs than snakes from McCafferty farm. Sex also had a significant effect on SVL, with adult females having greater SVLs than adult males (Table 2.4). A significant interaction was detected between sex and site (Table 2.4). Adult males from both sites were not significantly different in SVL ($F = 0.336$, $df = 1$, 95, $p = 0.563$), whereas adult females from Miller's marsh were significantly longer than adult females from McCafferty farm ($F = 54.81$, $df = 1, 247$, $p < 0.001$).

The significant interaction between sex and site for SVL is also apparent when an Index of Sexual Size Dimorphism (ISSD) is used (Gibbons & Lovich, 1990; Shine, 1993). For snakes, the ISSD is calculated by dividing the mean SVL of the larger sex by the smaller sex, and subtracting this ratio from one. Generally, the ISSD measurement is used to compare snakes at maturity (Shine, 1993). Overall, for my study the ISSD for adult males and females from the two sites combined was -0.19, which reflects a female biased size dimorphism. I also calculated separate SSD indices for the snakes from Miller's marsh and McCafferty farm. The ISSD for the snakes from Miller's marsh was -0.24, and the ISSD for the snakes from McCafferty farm was -0.14. The differences between the two indices reflect the significant sex by site interaction for SVL.

The mean body masses of all males from both sites were nearly identical (Table 2.3A, Figure 2.2A-B). Only adult males (SVL > 350.0 mm) at the two sites were compared statistically for differences in mass. Adult males captured at McCafferty farm were only slightly heavier than adult males at Miller's marsh (McCafferty's, M mass =

Table 2.4: Results of univariate ANOVA testing for site and sex effects on snout-vent length (SVL) in 346 wild-caught adult garter snakes.

Factor	df	MS	F	p
Site	1	0.18	17.84	<0.001
Sex	1	2.17	211.76	0.001
Site*Sex	1	9.74E-02	9.51	0.002
Error	342	1.02E-02		

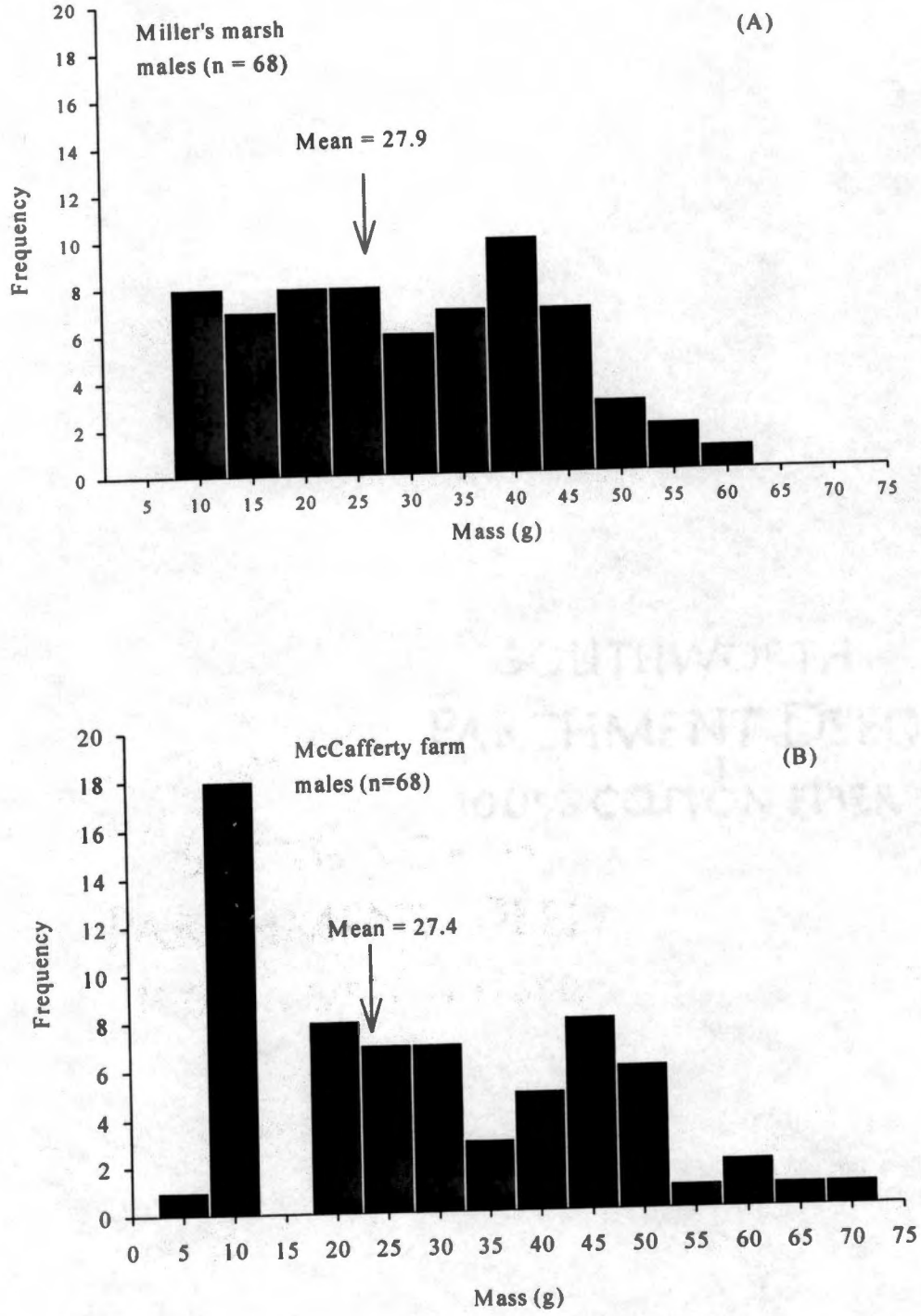
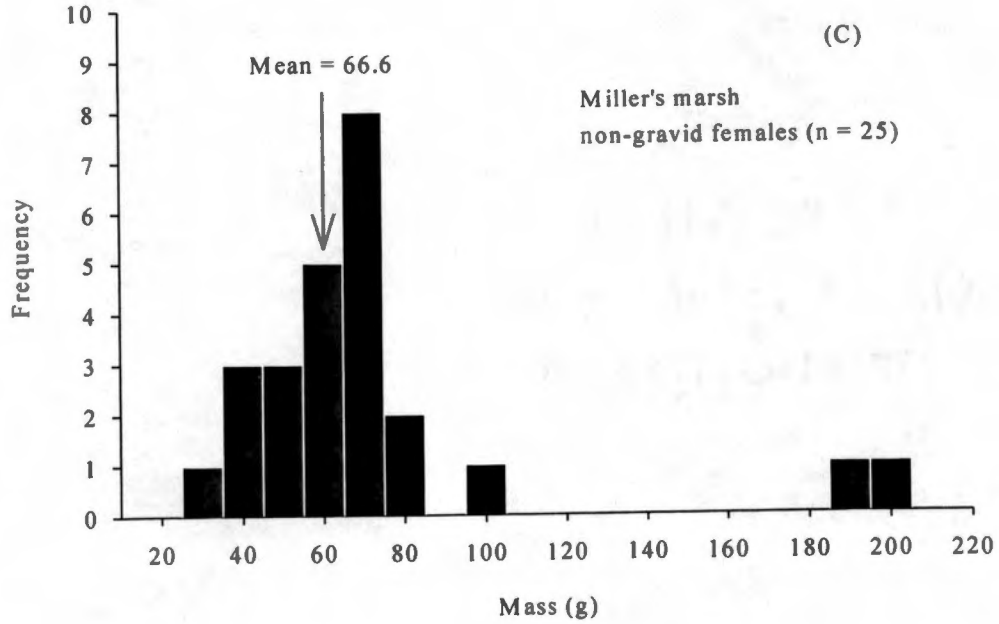


Figure 2.2: Mass frequency histograms for male snakes from Miller's marsh (A) and McCafferty farm (B), adult non-gravid females (SVL > 400.0 mm) from Miller's marsh C) and McCafferty farm (D), and for gravid females from Miller's marsh (E) and McCafferty farm (F).



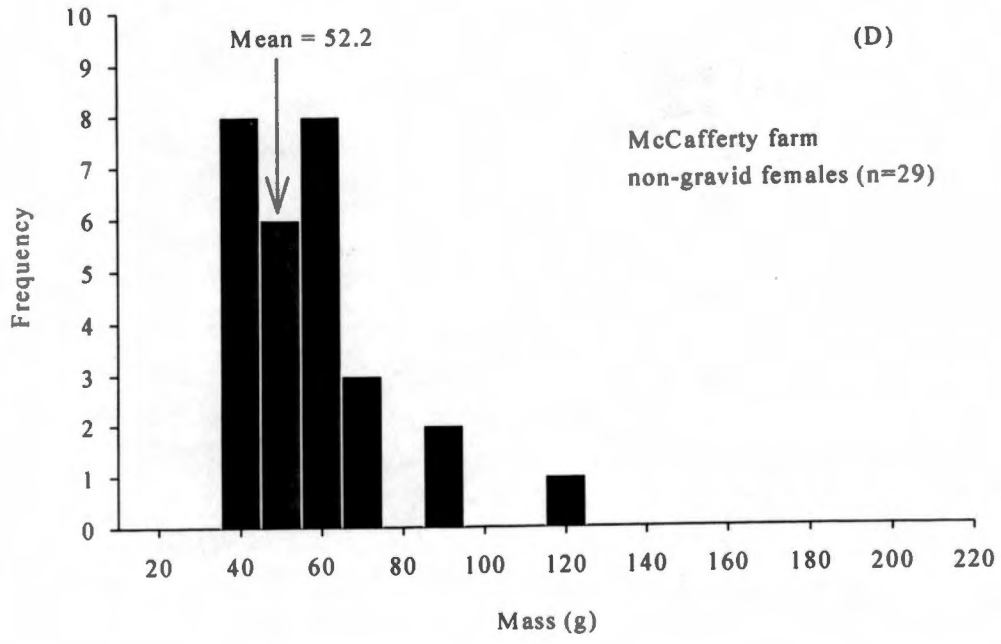


Figure 2.2 (continued)

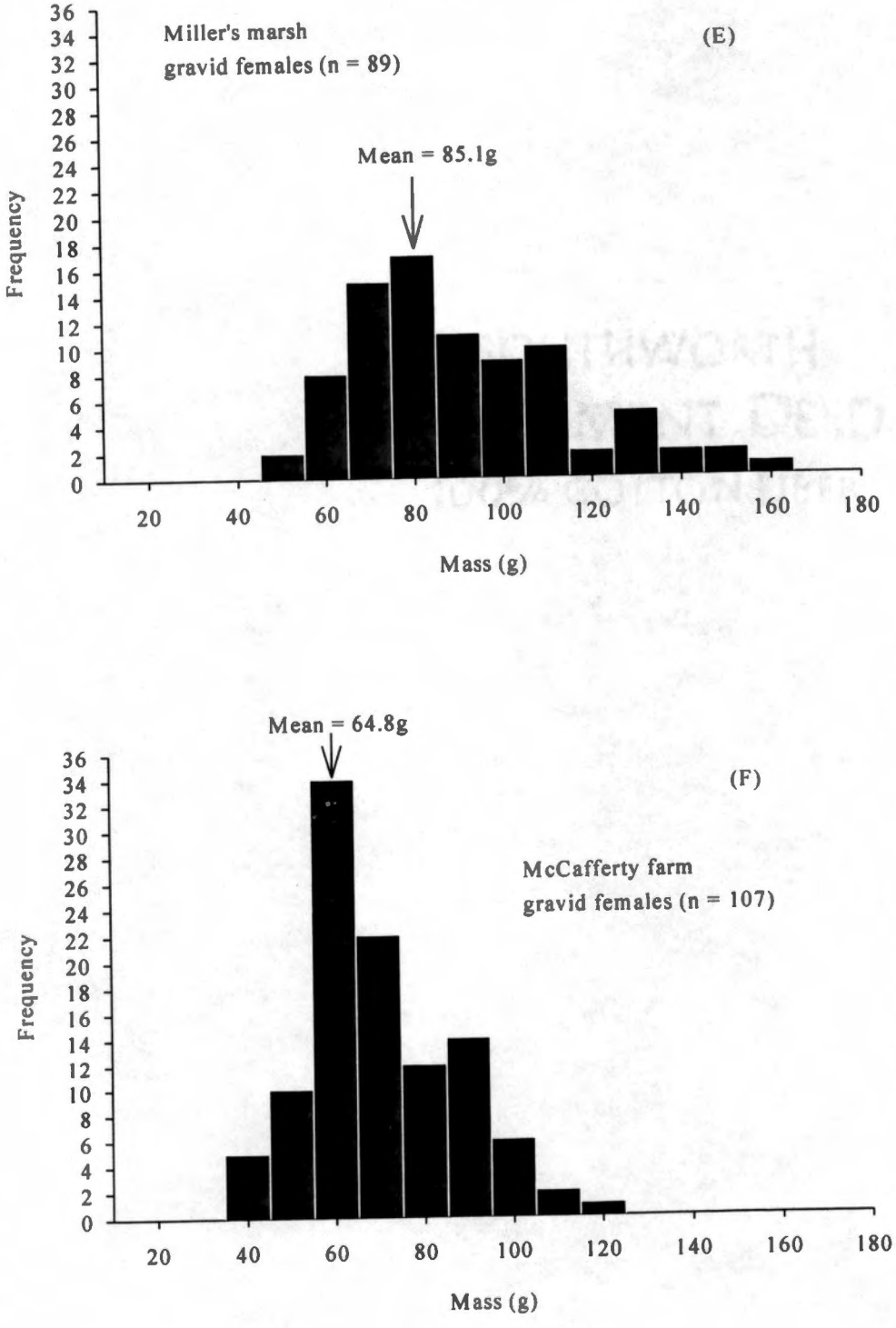


Figure 2.2 (continued)

36.6g; Miller's, M mass = 33.4g, Table 2.3B). However, with SVL treated as a covariate, males from McCafferty farm were significantly heavier than males from Miller's marsh (Table 2.5). Twenty-five non-gravid adult females were captured at Miller's marsh (M mass ± SE = 66.6 g ± 8.0) and 28 were captured at McCafferty farm (M mass ± SE = 52.2 g ± 3.58). Body weights between these two groups were significantly different (Table 2.5, Figure 2.2C-D). Non-gravid females from Miller's marsh also had significantly longer SVLs (M SVL ± SE = 527.4 mm ± 13.23) than non-gravid females from McCafferty farm (M SVL ± SE = 476.7 mm ± 12.02, $F = 8.08$, df = 1, 51, $p = 0.006$).

Gravid females from Miller's marsh were heavier (M mass ± SE = 85.1 g ± 2.54) than gravid females from McCafferty farm (M ± SE = 64.8 g ± 1.59), but the difference was not significant (Table 2.5, Figure 2.2E-F). However, with SVL removed as a covariate the body weights of gravid females differed significantly among sites ($F = 51.25$, df = 1, 194, $p < 0.001$). Gravid females from Miller's marsh were significantly longer (M SVL ± SE = 546.0 mm ± 5.01) than gravid females from McCafferty farm (M SVL ± SE = 500.8 mm ± 3.9, $F = 52.4$, df = 1, 194, $p < 0.001$).

Snout-vent length and mass showed significant linear relationships for all males and females from Miller's marsh and McCafferty farm (Table 2.6). Because the regression coefficients and slopes of the data from the two sites did not differ, the data were pooled. Using the pooled data, significant linear relationships between SVL and mass were again found for both males and females (Table 2.6; Figures 2.3A-B).

Table 2.5: Results of Univariate ANOVAs testing for site and sex effects on mass, with snout-vent length (SVL) as a covariate, in male, non-gravid female, and gravid female garter snakes captured at Miller's marsh and McCafferty farm.

Sex	Factor	df	MS	F	p
Males	SVL	1	9.06	546.0	<0.001
	Site	1	0.350	21.1	<0.001
	Error	94	1.66E-02		
Non-gravid females	SVL	1	6.09	208.13	<0.001
	Site	1	0.142	4.85	0.032
	Error	50	2.93E-02		
Gravid females	SVL	1	9.1	482.66	<0.001
	Site	1	5.94E-02	3.15	0.078
	Error	193	1.89E-02		

Note: Miller's marsh males, n = 52, McCafferty farm males, n = 45. Miller's marsh non-gravid females, n = 25, McCafferty farm non-gravid females, n = 28. Miller's marsh gravid females, n = 89, McCafferty farm gravid females, n = 107.

Table 2.6: Results of regression analyses of snout-vent length and mass in males and females from each site and in both sites combined.

Site	Sex	r^2	F	df	p
MM	Males	0.95	1200.9	1, 66	<0.001
	Females	0.96	3253.1	1, 141	0.001
MF	Males	0.98	3172.1	1, 134	0.001
	Females	0.97	4927.0	1, 176	0.001
Sites combined	Males	0.96	3172.1	1, 134	0.001
	Females	0.96	7907.2	1, 320	0.001

Note: MM = Miller's marsh, MF = McCafferty farm.

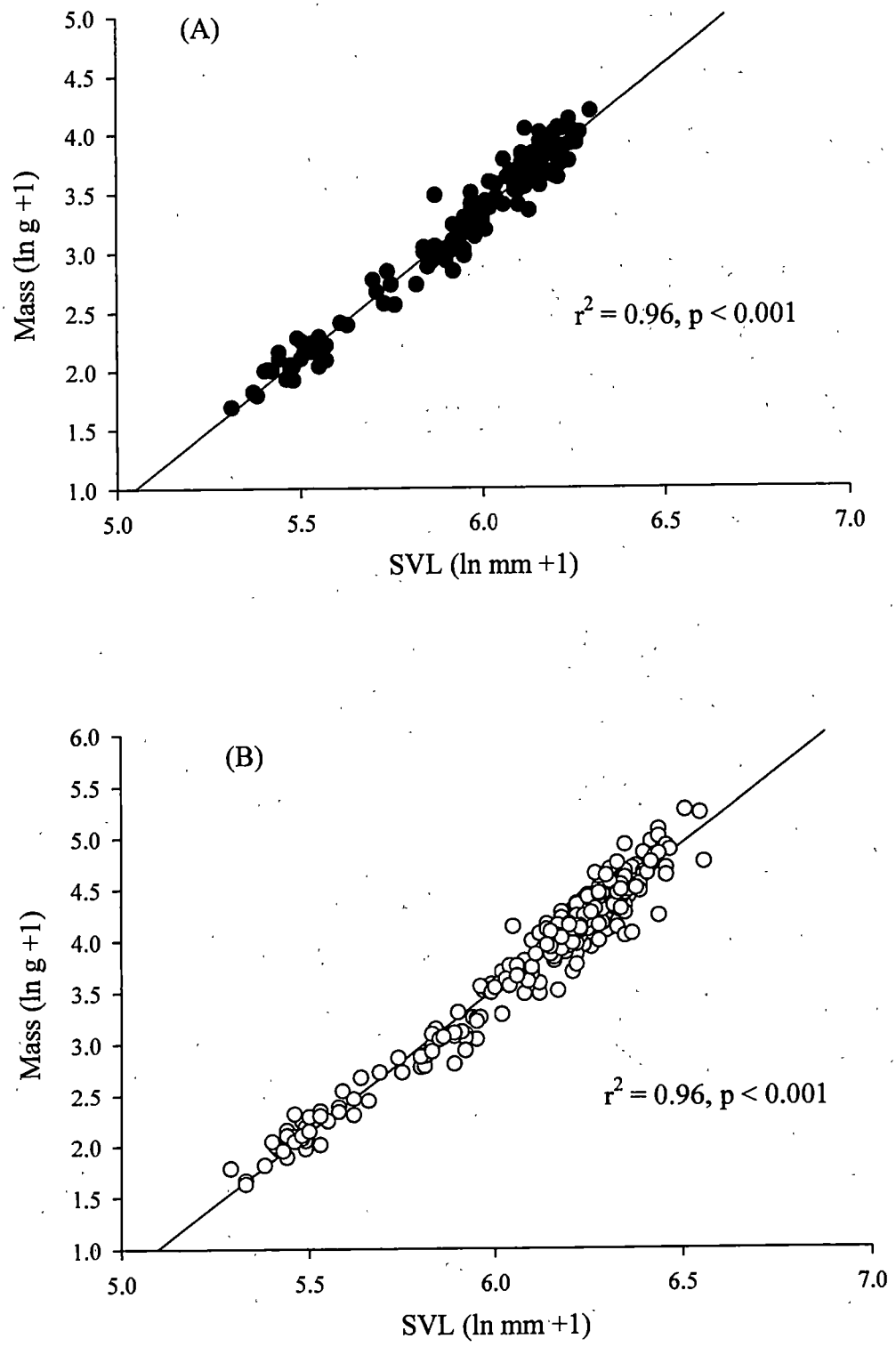


Figure 2.3: Relationship between mass and snout-vent length (SVL) in 136 male (A) and 321 female (B) wild-caught garter snakes. Regression equation for males is SVL = 4.7 + 0.39Mass and for females is SVL = 4.8 + 0.34Mass.

Phenotypic correlations

Phenotypic correlations among the four head measurements were computed (controlling for SVL). Significant phenotypic correlations among all four head measurements were found for females at both sites, and males from McCafferty farm (Table 2.7). The males from Miller's marsh showed significant phenotypic correlations between HL and JL ($p < 0.001$), HL and IOD ($p < 0.005$), HW and IOD ($p < 0.038$), HW and JL ($p < 0.022$), and JL and IOD ($p < 0.017$). However, after adjusting the test-wise significance level to 0.013 using Holm's sequentially rejective procedure (Aickin & Gensler, 1996), the relationships between the last three comparisons were not significant.

Site and sex differences in head size and shape

Descriptive statistics for raw values of HL, JL, HW, and IOD for snakes of each sex at both sites are shown in Table 2.3A. The initial MANCOVA including all snakes measured during the 1998 field season revealed no significant effect for site on relative head size ($\lambda = 0.988$, $F = 0.90$, $df = 4, 288$, $p = 0.464$). An overall effect was found for sex, with females having larger head sizes along length dimensions (HL and JL) than males ($\lambda = 0.792$, $F = 18.92$, $df = 4, 288$, $p < 0.001$, see Table 2.8, Figure 2.4A-D). Sex and site did not interact ($\lambda = 0.989$, $F = 0.810$, $df = 4, 288$, $p = 0.519$, see Table 2.8). Snout-vent length covaried significantly with HL, JL, HW, and IOD ($F = 1404.29$, $df = 4, 288$, $p < 0.001$).

Age effects may have biased tests for differences in head sizes among sites. If phenotypic plasticity were to account for the hypothesized head size variation, then differences would probably not appear in very young snakes. Therefore, tests for site

Table 2.7: Phenotypic correlations between four head measurements in 295 wild-caught, adult garter snakes from Miller's marsh and McCafferty farm.

	MM				MF		
		Males (n=32)				Males (n=43)	
	JL	HW	IOD		JL	HW	IOD
HL	0.754**	0.381	0.487**	HL	0.88**	0.696**	0.642**
JL		0.41	0.425	JL		0.738**	0.643**
HW			0.374	HW			0.679**
		Females (n=97)				Females (n=123)	
HL	0.923**	0.682**	0.499**	HL	0.816**	0.631**	0.488**
JL		0.715**	0.457*	JL		0.682**	0.501**
HW			0.534**	HW			0.613**

Note: MM=Miller's marsh, MF=McCafferty farm. All *p* values are two-tailed after controlling for multiple comparisons.

** = $p < 0.001$

* = $p < 0.01$

Table 2.8: MANCOVA results testing for site and sex differences in head length (HL), jaw length (JL), head width (HW), and inter-ocular distance (IOD) in 296 wild-caught garter snakes. Significant p-values are boldfaced.

Source of variation	DV	df	MS	F	p
Covariate (SVL)	HL	1	9.45	4426.55	**<0.001**
	JL	1	12.17	5221.37	**0.001**
	HW	1	6.05	1794.13	**0.001**
	IOD	1	6.56	2162.98	**0.001**
Site	HL	1	1.23E-04	0.06	0.811
	JL	1	8.36E-04	0.36	0.550
	HW	1	1.57E-05	0.01	0.945
	IOD	1	4.65E-03	1.53	0.217
Sex	HL	1	4.58E-02	21.47	**<0.001**
	JL	1	0.141	60.64	**0.001**
	HW	1	5.89E-02	17.46	**0.001**
	IOD	1	1.48E-02	4.89	**0.028**
Site by Sex	HL	1	1.93E-03	0.90	0.343
	JL	1	5.89E-03	2.53	0.113
	HW	1	4.35E-03	1.29	0.257
	IOD	1	5.82E-04	0.19	0.662
Error	HL	291	2.14E-03		
	JL	291	2.33E-03		
	HW	291	3.37E-03		
	IOD	291	3.03E-03		

Note: Miller's marsh: males, n = 32; females, n = 97. McCafferty farm: males, n = 43, females, n = 124.

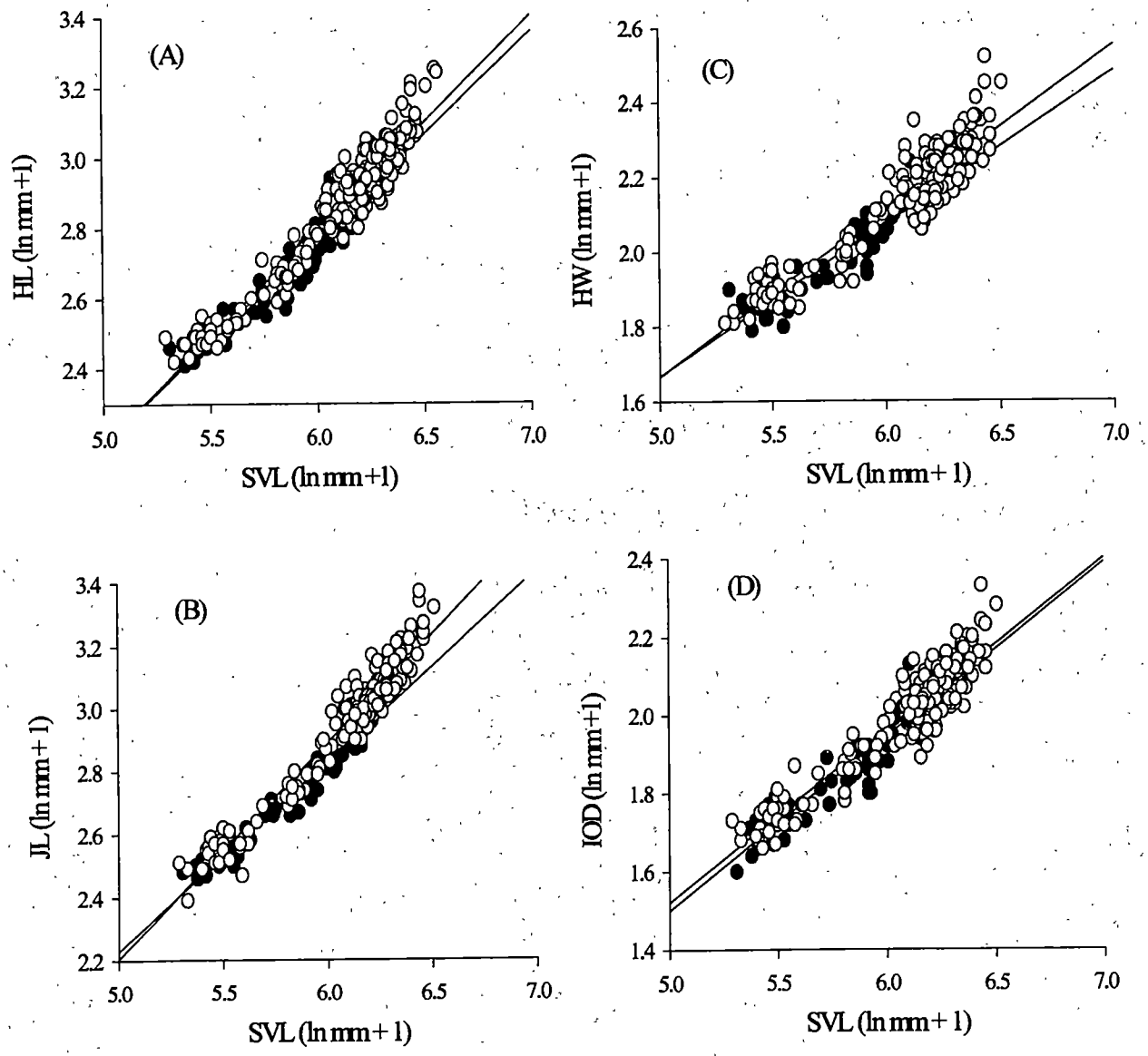


Figure 2.4: Relationships between snout-vent length (SVL) and head length, HL (A), jaw length, JL (B), head width, HW (C), and inter-ocular distance, IOD (D) in male (closed circles, n = 75) and female (open circles, n = 221) wild-caught garter snakes.

effects were repeated using only adult snakes (male SVL > 350.0 mm; female SVL > 400.0 mm). Descriptive statistics for the adults tested in this second MANCOVA are shown in Table 2.3B. With the sub-adult snakes removed, site had a significant effect on head size ($\lambda = 0.94$, $F = 3.21$, $df = 4, 200$, $p = 0.014$, see Table 2.9), as did sex ($\lambda = 0.798$, $F = 12.68$, $df = 4, 200$, $p < 0.001$). Sex and site did not interact for adult snakes ($\lambda = 0.992$, $F = 0.28$, $df = 4, 200$, $p = 0.823$). Snout-vent length covaried significantly with all head measurements ($\lambda = 0.255$, $F = 146.14$, $df = 4, 200$, $p < 0.001$) and significant sex differences were found for HL and JL. The site effect was primarily accounted for by IOD (Table 2.9).

Method: Postpartum Morphology

Seventeen gravid females from Miller's marsh and 16 gravid females from McCafferty farm were captured and brought to the University of Tennessee following the 1998 (n = 22 litters) and 1999 (n = 11 litters) field seasons. The females were housed separately and fed either minnows (*Pimephales promelas*) or earthworms (*Lumbricus terrestris*) once per week (depending on availability), and water was available ad libitum. Room temperature was 25° C, relative humidity was 30%, and a 12:12 light:dark cycle was maintained. Neonates from 19 of 22 litters born during 1998 (n = 146) were used to study the effects of diet on growth rates. (The litters used in both analyses are listed in the Appendix I). After giving birth, adult females were returned to Beaver Island each Fall.

Table 2.9: MANCOVA results testing for site and sex differences in head length (HL), jaw length (JL), head width (HW), and inter-ocular distance (IOD) in 208 adult garter snakes. Significant p-values are boldfaced.

Source of variation	DV	df	MS	F	p
Covariate (SVL)	HL	1	0.91	413.08	**<0.001**
	JL	1	1.18	532.97	**0.001**
	HW	1	0.60	162.16	**0.001**
	IOD	1	0.71	216.16	**0.001**
Site	HL	1	1.16E-03	0.53	0.469
	JL	1	2.22E-05	0.01	0.920
	HW	1	1.57E-04	0.04	0.837
	IOD	1	2.41E-02	7.38	**0.007**
Sex	HL	1	9.93E-03	4.47	**0.035**
	JL	1	5.38E-02	24.29	**0.001**
	HW	1	1.10E-02	2.99	0.085
	IOD	1	2.20E-04	0.07	0.795
Site by Sex	HL	1	2.21E-03	0.00	0.996
	JL	1	2.22E-03	0.03	0.854
	HW	1	3.69E-03	0.03	0.867
	IOD	1	3.27E-03	0.50	0.480
Error	HL	203	2.21E-03		
	JL	203	2.22E-03		
	HW	203	3.69E-03		
	IOD	203	3.27E-03		

Note: Miller's marsh: males, n = 20; females, n = 79. McCafferty farm: males, n = 20; females, n = 89.

Subjects and maintenance

From birth, the neonates were housed separately in clear plastic cages (13.5 x 18.5 x 4.0 cm, or 12.0 x 17.0 x 9.0 cm), each including a cardboard substrate, shelter, and water dish. Room temperature was kept constant (25° C), with 30% relative humidity, and a 12:12-hr light:dark cycle was maintained throughout the study period. Cages were cleaned as needed and water was available ad libitum.

Procedure

Within 24 h of birth, mass, SVL, TL, HL, JL, HW, and IOD were measured on all neonates using the same protocol as for adults. Body weights (to the nearest 0.01g) were obtained using a digital scale, SVL and TL (to the nearest 1.0 mm) were measured using a meter stick, and head measurements (to nearest 0.01 mm) were made with a hand-held digital caliper. Sex was determined by cloacal probing. JL, HW, and IOD were not measured on neonates during the 1999 season. Multivariate analyses on head morphology are thus restricted to 156 of the neonates born in 1998.

Statistical analyses

Sex, litter, and site differences in neonatal SVL, mass, and TL were tested with ANOVA, with sex and site specified as fixed factors, and litter as a random factor nested within site. Snout-vent length was treated as a covariate for comparisons of mass and TL. Head measurements were analyzed using MANCOVA (covariate = SVL) with sex and site treated as fixed factors, and litter nested in site as a random factor. Multivariate significance for SVL, sex, site, and litter was tested using Wilk's Lambda, and effects for each of these factors on all four head measurements were tested with univariate F-tests. Phenotypic correlations between HL, JL, HW, and IOD were determined using partial

correlation coefficients, with SVL and litter factored out. Relationships between maternal SVL and litter size, and mean neonatal SVL, and mass were tested using simple linear regression. All data were normalized using natural log (+1) transformations.

Results: Postpartum Morphology

A total of 286 neonates was born over the two years (Table 2.10). Comparisons by sex, site, and litter for SVL, tail, and mass were made with all 286 snakes. Descriptive statistics (M ± 1SE) on mass, SVL, TL, HL, JL, HW, and IOD at birth for snakes of each sex, and site are shown in Table 2.11. Descriptive statistics for all postpartum measurements for each litter can be found in Appendix II.

Litter, sex, and site differences in body size

Significant litter effects were found for neonatal mass, SVL, and TL (Table 2.12). Males and females differed in all three measurements, with males having greater SVLs and TLs, and females having greater body weights (Table 2.12). At birth, neonates born to mothers collected at Miller's marsh were slightly heavier and had longer SVLs than neonates from McCafferty farm, but the differences were not significant and no site effect was found for TL. Mass and SVL were significantly correlated at birth for both males and females. The linear relationship between snout-vent length and mass was significant for both males and females (Figure 2.5).

Maternal correlations

Maternal SVL did not significantly predict mean litter sizes for the 33 litters born during the 1998 and 1999 seasons (Figure 2.6). Maternal SVL also did not predict neonatal SVL (Figure 2.7A) and only a marginal relationship was found between maternal SVL and mean neonatal body mass (Figure 2.7B). Nevertheless, all of the

Table 2.10: Litter sizes and frequencies of each sex for snakes born to mothers from Miller's marsh and McCafferty farm during 1998 and 1999. These snakes were used in analyses for litter, sex, and site differences in snout-vent length, tail length, and mass at birth.

Site	n	# litters	M (SD) Litter size	Range	Males (n)	Females (n)
MM	175	17	10.3(3.4)	3-17	84	91
MF	111	16	6.9(2.8)	2-13	58	53
Totals	286	33	8.7(3.5)	2-17	142	144

Note: MM = Miller's marsh, MF = McCafferty farm.

Table 2.11: Descriptive statistics (M ± 1SE) by site and sex for mass, snout-vent length (SVL), tail length (TL), head length (HL), jaw length (JL), head width (HW), and inter-ocular distance (IOD) for neonates born in 1998 and 1999 to mothers from McCafferty farm (MF) and Miller's marsh (MM).

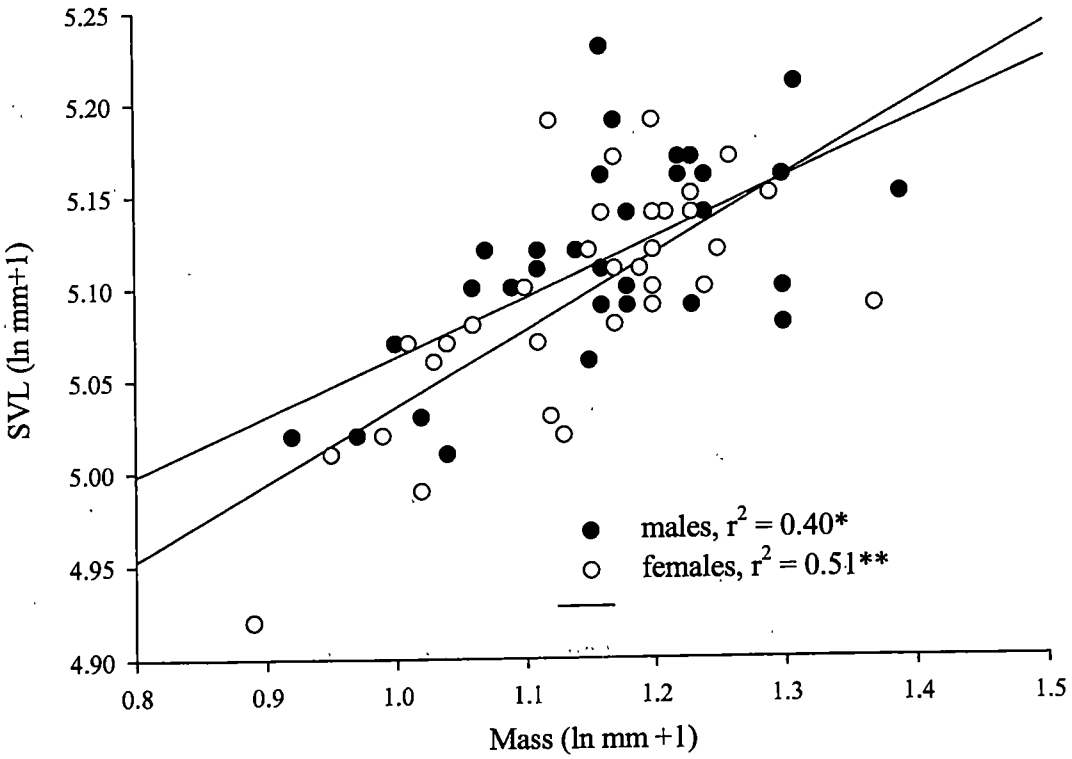
Site	Sex	Mass (0.1g)	SVL (1.0mm)	Tail (1.0mm)	HL (0.01mm)	JL (0.01mm)	HW (0.01mm)	IOD (0.01mm)
98/99MF Totals	M	2.07(0.04)	162.3(1.04)	48.9(0.51)	9.98(0.05)	9.80(0.05)	4.54(0.03)	4.00(0.02)
	F	2.16(0.04)	161.9(1.30)	45.0(0.60)	10.13(0.06)	10.06(0.11)	4.63(0.03)	4.07(0.03)
	Total	2.11(0.03)	162.1(0.82)	47.1(0.43)	10.05(0.04)	9.90(0.06)	4.58(0.02)	4.03(0.02)
98/99MM Totals	M	2.22(0.05)	166.1(1.14)	48.3(0.47)	10.04(0.04)	9.94(0.05)	4.61(0.02)	4.04(0.02)
	F	2.23(0.04)	162.7(1.08)	45.5(0.54)	10.20(0.04)	10.13(0.04)	4.69(0.02)	4.09(0.02)
	Total	2.23(0.03)	164.3(0.79)	46.8(0.37)	10.13(0.03)	10.05(0.03)	4.65(0.01)	4.07(0.01)
Grand Totals	M	2.16(0.03)	164.5(0.81)	48.6(0.35)	10.02(.03)	9.88(0.04)	4.58(0.02)	4.02(0.02)
	F	2.21(0.03)	162.4(0.83)	45.3(0.40)	10.17(0.04)	10.11(0.04)	4.67(0.02)	4.09(0.02)
	Total	2.18(0.02)	163.5(0.58)	46.9(0.28)	10.10(0.02)	9.99(0.03)	4.62(0.01)	4.06(0.01)

Note: JL, HW, and IOD were not measured for 1999 neonates. Head measurements for two litters born late in 1998 were not taken.

Table 2.12: Results of univariate ANOVAs testing for sex and site effects on neonatal snout-vent length (SVL), mass, and tail length (TL).

Measure	Source	df	MS*	F	p
SVL					
	Litter within Site	31, 251	2.38E-02	19.75	**<0.001**
			1.21E-03		
	Sex	1, 251	2.16E-02	17.86	**0.001**
			1.21E-03		
	Site	1, 32	1.34E-02	0.70	0.408
			1.90E-02		
Mass	Covariate (SVL)	1, 250	0.46	180.23	**<0.001**
			2.54E-03		
	Litter within Site	31, 250	4.78E-02	18.83	**0.001**
			2.54E-03		
	Sex	1, 250	1.16E-02	4.59	**0.033**
			2.54E-03		
	Site	1, 32	4.99E-02	1.33	0.258
			3.76E-02		
TL	Covariate (SVL)	1, 250	0.18	64.99	**<0.001**
			2.81E-03		
	Litter within Site	31, 250	3.61E-02	12.85	**0.001**
			2.81E-03		
	Sex	1, 250	0.22	78.62	**0.001**
			2.81E-03		
	Site	1, 32	2.25E-02	0.79	0.382
			2.86E-02		

* MS = Hypothesis Mean Squared Error above MS(error)



* F = 20.52, df = 1, 31, p < 0.001
** F = 32.57, df = 1, 31, p < 0.001

Figure 2.5: Relationship between snout-vent length (SVL) and Mass in neonatal garter snakes (n = 33 litters). Litter means for each sex are plotted. Regression equation for males is SVL = 4.74 + 0.32Mass, and females is SVL = 4.62 + 0.41Mass.

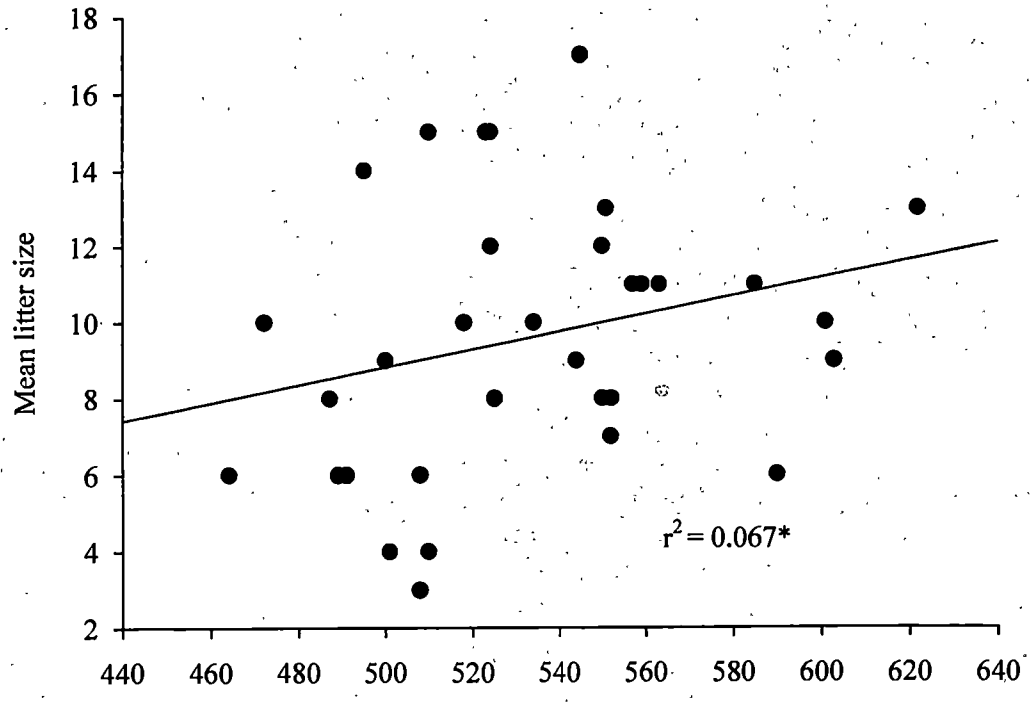
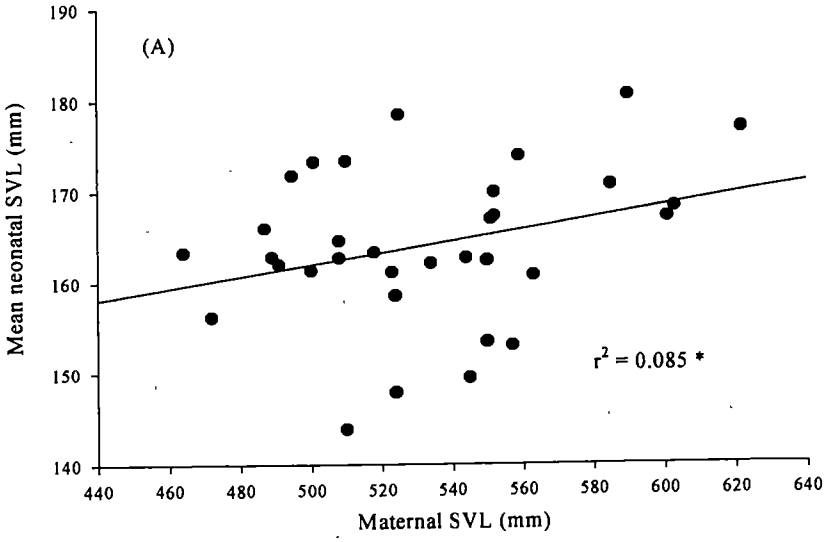
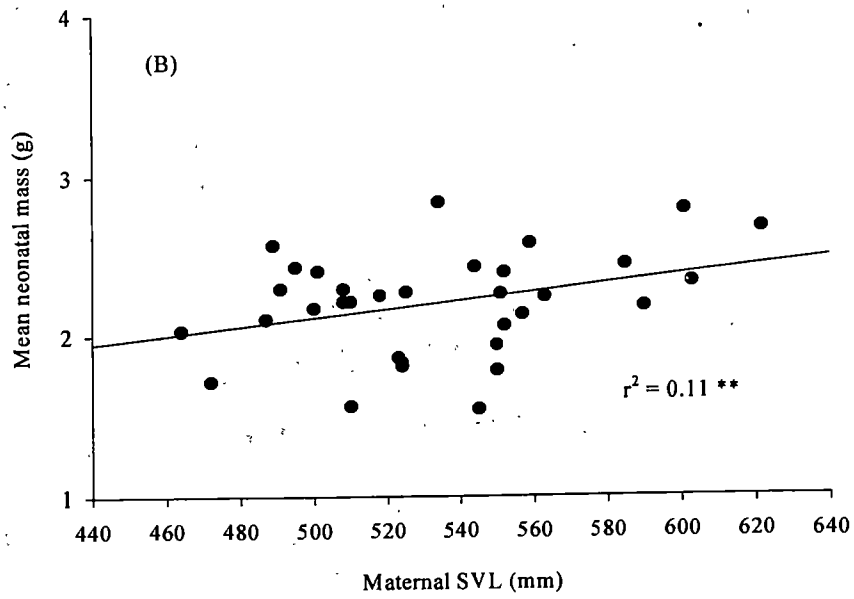


Figure 2.6: Relationship between litter size (n=33) and maternal snout-vent length (SVL) in garter snakes from both sites. Regression equation is Litter size = -2.87 + 0.02SVL.





*F = 2.87, df = 1, 32, p = 0.10
**F = 3.66, df = 1, 32, p = 0.065

Figure 2.7: Relationship between mean neonatal and maternal snout-vent length, SVL (A), and mean neonatal mass and maternal SVL (B) in 33 garter snake litters. Regression equations are neonatal SVL = 129.7 + 0.06SVL, and neonatal mass = 2.71 + 0.75SVL.

slopes were positive, suggesting that larger females have a tendency to have more and larger offspring.

Phenotypic correlations

Phenotypic correlations among the four head measurements were computed with SVL and litter factored out. At birth, there were significant positive phenotypic correlations between the four head measurements (see Table 2.13). All correlations were significant at $p < 0.001$. Adjusting significance levels for the six correlations using the Bonferroni procedure (0.05/6 = 0.008) and Holm's sequentially rejective procedure did not change the outcome of the tests.

Head size variation

Snout-vent length covaried significantly with the four head measurements (λ = .595, $F = 19.21$, $df = 4, 113$, $p < 0.001$). The overall effect for site on neonatal head size was not significant ($\lambda = 0.58$, $F = 2.53$, $df = 4, 14$, $p = 0.087$), but snakes from Miller's marsh had significantly greater JLs and IODs (see Table 2.11 and 2.14). There was a significant overall effect for sex ($\lambda = 0.422$, $F = 4.80$, $df = 4, 14$, $p = 0.012$), with females having greater JLs, HLs, HWs, and IODs than males (Table 2.14). The overall effect for litter was significant ($\lambda = 0.283$, $F = 2.49$, $df = 68, 464$, $p < 0.001$), with all four head measurements showing significant litter variation (Table 2.14). The litter by sex and sex by site interactions were not significant for any of the head measurements.

Table 2.13: Phenotypic correlations between four head measurements in155 neonatal garter snakes.

	Jaw length	Head width	Inter-ocular distance
Head length	0.497**	0.495**	0.334**
Jaw length		0.446**	0.365**
Head width			0.556**

Note: All p values are two-tailed after controlling for multiple comparisons.
** = $p < 0.001$

Table 2.14: Results of MANCOVA testing for site, sex, and litter effects on head length (HL), jaw length (JL), head width (HW), and inter-ocular distance (IOD) in 155 neonatal garter snakes.

Source of variation		df	Hypothesis MS	Error MS	F	p
Covariate (SVL)	HL	1	2.15E-02	6.0E-04	35.69	**<0.001**
	JL	1	4.76E-02	7.0E-04	67.63	**0.001**
	HW	1	1.10E-02	5.7E-04	19.22	**0.001**
	IOD	1	1.12E-02	6.9E-04	16.26	**0.001**
		116				
Site	HL	1	1.83E-03	1.52E-03	1.21	0.287
	JL	1	1.67E-02	2.25E-03	7.41	**0.015**
	HW	1	2.0E-03	1.17E-03	1.71	0.208
	IOD	1	1.55E-02	2.97E-03	5.22	**0.035**
		17				
Sex	HL	1	3.79E-03	6.0E-04	5.71	**0.029**
	JL	1	2.05E-02	7.0E-04	22.23	**0.001**
	HW	1	8.29E-03	5.7E-04	12.25	**0.003**
	IOD	1	3.20E-03	6.9E-04	8.45	**0.010**
		17				
Litter within Site	HL	17	1.52E-03	6.0E-04	2.52	**0.002**
	JL	17	2.25E-03	7.0E-04	3.20	**0.001**
	HW	17	1.17E-03	5.7E-04	2.05	**0.013**
	IOD	17	2.97E-03	6.9E-04	4.30	**0.001**
		116				
Litter by Sex	HL	17	6.6E-04	6.0E-04	1.10	0.363
	JL	17	9.2E-04	7.0E-04	1.31	0.198
	HW	17	6.8E-04	5.7E-04	1.19	0.286
	IOD	17	3.8E-04	6.9E-04	0.55	0.922
		116				
Sex by Site	HL	1	2.2E-04	6.0E-04	0.36	0.548
	JL	1	5.0E-05	7.0E-04	0.07	0.785
	HW	1	4.6E-04	5.7E-04	0.80	0.373
	IOD	1	1.1E-4	6.9E-04	0.16	0.160
		116				

Method: Diet and Growth

Subjects and maintenance

Subjects were 146 neonates born to 19 gravid females (M litter size = 8.2, range = 2 - 15, see Appendix I) collected at Miller's marsh (n = 9 litters) and McCafferty farm (n = 10 litters). These snakes were also included in the postpartum morphological studies and their housing conditions are the same as above.

Procedure

The snakes were assigned to one of three feeding conditions: Fish (F group, n = 48), Worm (W group, n = 49), and Mixed (FW group, n = 48). The fish (*Pimephales promelas*) and the worms (*Lumbricus rubellus*) were purchased from commercial suppliers. The fish ranged in size from 0.14 g to 0.38 g and the worms ranged from 0.17 g to 0.36 g. Diet assignments were balanced as well as possible across litters, sexes (78 males; 78 females), and sites (Miller's marsh = 97; McCafferty farm = 59). Color-coded labels were placed on the outside of each snake's cage to designate its diet group.

During feedings, live fish were placed in each snake's water dish. Worms were placed in shallow petri dishes layered with dirt. The snakes were offered their first meal at 20 days of age. SVL, mass and TL measurements were taken at birth, 80 days, 160 days, and 240 days. Head measurements were taken at birth, 160 days, and 240 days. The snakes were fed once weekly on their respective diets between 20 and 160 days, and twice weekly between 160 and 240 days (to meet increased dietary needs). This study was combined with another that investigated the role of experience and memory on prey handling abilities (see Chapter 4). The procedure required switching the prey for the F and W groups at 160 days of age. Thus the data between 160 and 240 days represent

changes in growth after diets were switched for these two groups. The FW group remained on the mixed diet for the entire 240-day period. When the study was completed, the snakes were scale-clipped for identification and released at their respective sites on Beaver Island.

Statistical analyses

To test for differences in growth (SVL and mass) across time and diet effects on growth, data were analyzed using a MANOVA method for repeated measures (O'Brian & Kaiser, 1985). Dependent variables were thus treated as the differences in the linear dimensions between measurements, with three degrees of freedom. Diet was treated as a fixed factor, and a significant effect would indicate an interaction between diet and growth across time. To determine which of the three groups differed, significant interactions were subsequently analyzed with separate univariate F-tests. Separate MANCOVAs for repeated measures were run for SVL and mass. The effects of diet on relative head size (HL, JL, HW, and IOD) were tested separately with ANCOVA at birth, 160 and 240 days. Sex, site, and diet were treated as fixed factors, and SVL at each corresponding age was treated as a covariate. Because of high subject mortality, litter was not included as a factor at 160 and 240 days. All data were natural log transformed.

Results: Diet and Growth

Feeding records indicated that similar proportions of fish and worms were eaten by snakes in all diet groups. The snakes in the F group consumed 88.2% of the fish offered (M = 24.2 fish per snake). The W group consumed 88.6% (M = 23.4) of the worms. The FW group consumed 91.3% of fish (M = 11.3) and 87.2% of worms (M = 13.7). The mean (+1SE) increases of litter SVL and mass for each diet group are shown

in Figures 2.8A and 2.8B respectively (see Appendix III for descriptive statistics). Site was not a significant factor for increases in SVL ($F = 0.64$, $df = 3, 30$, $p = 0.594$) or mass ($F = 0.02$, $df = 3, 30$, $p = 0.997$) at any point of the study. Data were combined by site for analyses of SVL and mass. Snake mortality greatly reduced the sample sizes within the diet groups. Therefore, tests for litter effects were excluded. Sample sizes used in the repeated measures MANOVA were 10 snakes (F group), 13 snakes (W group), and 11 snakes (FW group).

Diet effects on snake body size

At birth, SVL did not differ among the three diet groups ($F = 0.30$, $df = 2, 142$, $p = 0.742$), indicating that there were no pre-existing size biases prior to feeding (Figure 2.8A). A repeated measures ANOVA showed that SVL increased significantly with age for all diet groups ($F = 75.43$, $df = 3, 29$, $p < 0.001$). The repeated measures MANOVA testing for diet and sex effects on SVL at birth, 80, 160, and 240 days (Table 2.15) shows that diet had a significant overall effect on growth ($\lambda = 0.584$, $F = 2.67$, $df = 6, 52$, $p = 0.025$), and a marginally significant effect was found for sex ($\lambda = 0.752$, $F = 2.87$, $df = 3, 26$, $p = 0.056$).

Results from the separate F-tests comparing SVLs between birth, 80, 160 and 240 days (Table 2.15) were not statistically different among the three diet groups at 80 or 160 days. Marginally significant increases in SVL due to diet occurred when the snakes reached 240 days (0.055). This difference was due to the greater SVLs of the snakes in the FW group compared to the W group ($p = 0.05$).

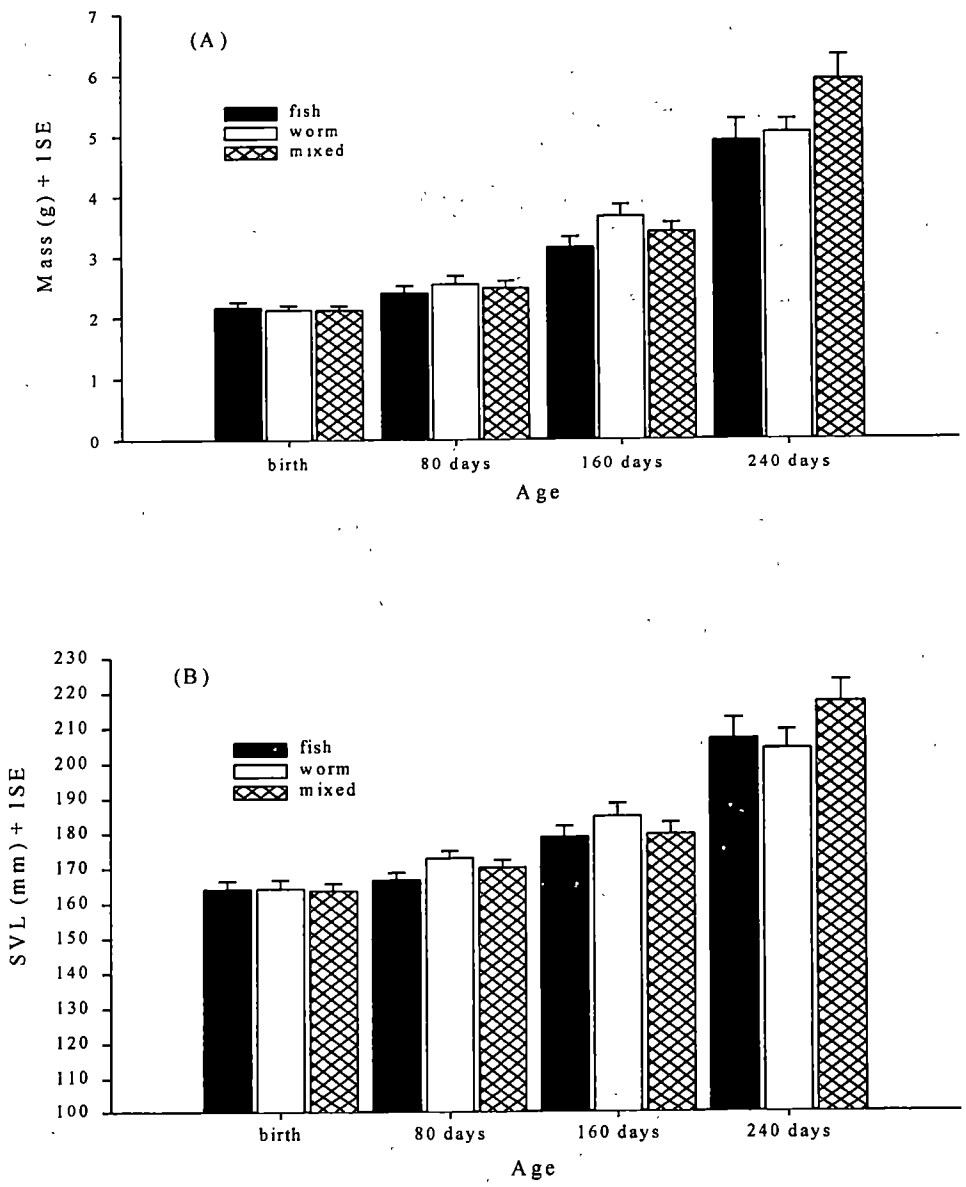


Figure 2.8: Mean (+ SE) snout-vent length, SVL (A) and mass (B) increases from birth to 240 days in garter snakes (n = 19 litters) on three diets.

Table 2.15: Results of repeated measures MANOVA testing for diet and sex effects on snout-vent length (SVL) increases from birth through 80, 160, and 240 days.

Source	DV	df	MS	F	p
Diet	birth vs 80	2	4.22E-05	0.03	0.972
	birth vs 160	2	3.49E-04	0.11	0.893
	birth vs 240	2	1.99E-02	3.23	0.055
Sex	birth vs 80	1	1.57E-04	0.11	0.745
	birth vs 160	1	8.35E-03	2.72	0.111
	birth vs 240	1	1.96E-02	3.17	0.086
Diet*Sex	birth vs 80	2	3.49E-04	0.24	0.789
	birth vs 160	2	5.43E-04	0.18	0.839
	birth vs 240	2	6.36E-03	1.03	0.370
Error	birth vs 80	28	1.46E-03		
	birth vs 160	28	3.08E-03		
	birth vs 240	28	6.17E-03		

Mass increased significantly with age (repeated measures ANOVA: $F = 104.34$, $df = 3, 29$, $p < 0.001$), but diet had no overall effect on the relative increases in mass among the snakes in the three groups ($\lambda = 0.694$, $F = 1.75$, $df = 6, 52$, $p = 0.131$). Sex also had no effect on relative mass increases ($\lambda = 0.850$, $F = 1.53$, $df = 3, 26$, $p = 0.231$). Marginally significant Sex differences between birth and 80 days (0.053), and birth and 160 days (0.052) were found (Table 2.16). Mass increases between birth and 240 days were not significant.

Diet effects on snake head size

Snout-vent length covaried significantly with HL, JL, HW, and IOD at birth, 160 days and 240 days (see Table 2.17A-C). Neither diet nor site had any effect on the relative size of HL, JL, HW, or IOD at any point of the study. The non-significant site effect at birth (Table 2.17A) contrasts with the significant site effects found for JL and IOD reported in Table 2.14, which included a larger sample of neonates. Sex had a significant effect on all measurements at all times except for HW and IOD at 240 days, with females having significantly larger relative head sizes than males at all ages, from birth through 240 days (see Figure 2.9A-D). Sex and diet did not interact at any time and for any of the four dependent variables. Sex did not have a significant effect on head growth rate ($F = 1.48$, $df = 3, 30$, $p = 0.241$).

Discussion

Diet induced variation in body size is relatively widespread among snakes, although genetic factors are known to account for geographic variation of morphological traits in snakes, as well as in other taxa (see Madsen & Shine, 1993, refs. therein). Phenotypic plasticity of body and head sizes has been found within many species of

Table 2.16: Results of repeated measures MANOVA testing for diet and sex effects on mass increases from birth through 80, 160, and 240 days.

Source	DV	df	MS	F	p
Diet	Birth vs 80	2	3.16E-02	2.94	0.069
	Birth vs 160	2	4.82E-02	3.06	0.063
	Birth vs 240	2	7.43E-02	1.35	0.276
Sex	Birth vs 80	1	4.38E-02	4.08	**0.053**
	Birth vs 160	1	6.48E-02	4.11	**0.052**
	Birth vs 240	1	2.65E-02	0.48	0.494
Diet*Sex	Birth vs 80	2	5.92E-04	0.95	0.946
	Birth vs 160	2	2.25E-03	0.87	0.868
	Birth vs 240	2	6.98E-02	0.30	0.297
Error	Birth vs 80	28	1.07E-02		
	Birth vs 160	28	1.58E-02		
	Birth vs 240	28	5.51E-02		

Table 2.17: MANCOVA results for tests of diet, sex, and site effects on head length (HL), jaw length (JL), head width (HW), and inter-ocular distance (IOD) at birth (A), at 160 days (B), and at 240 days (C).

(A) At birth

Source of variation	DV	df	MS	F	p
Covariate (SVL)	HL	1	2.30E-02	32.92	**<0.001**
	JL	1	4.03E-02	50.08	**0.001**
	HW	1	1.02E-02	18.98	**0.001**
	IOD	1	3.13E-03	4.16	**0.043**
Diet	HL	2	1.36E-04	0.20	0.823
	JL	2	5.61E-04	0.70	0.499
	HW	2	1.23E-04	0.23	0.793
	IOD	2	6.54E-05	0.09	0.917
Sex	HL	1	8.15E-03	11.67	**0.001**
	JL	1	2.61E-02	32.51	**<0.001**
	HW	1	1.13E-02	21.13	**<0.001**
	IOD	1	6.73E-03	8.95	**0.003**
Site	HL	1	1.55E-03	2.22	0.139
	JL	1	2.16E-04	0.27	0.605
	HW	1	1.99E-03	3.72	0.056
	IOD	1	4.19E-04	0.56	0.457
Error	HL	139	6.98E-04	32.92	
	JL	139	8.04E-04	50.08	
	HW	139	5.35E-04	18.98	
	IOD	139	7.53E-04	4.16	

Table 2.17 (continued)

(B) At 160 days

Source of variation	DV	df	MS	F	p
Covariate (SVL)	HL	1	1.76E-02	21.27	**<0.001**
	JL	1	1.42E-02	21.57	**<0.001**
	HW	1	7.35E-03	10.38	**0.002**
	IOD	1	1.44E-02	11.83	**0.001**
Diet	HL	2	7.51E-04	0.91	0.408
	JL	2	1.08E-04	0.16	0.850
	HW	2	6.01E-04	0.85	0.432
	IOD	2	7.18E-05	0.06	0.943
Sex	HL	1	1.69E-02	20.43	**<0.001**
	JL	1	1.25E-02	19.01	**<0.001**
	HW	1	7.54E-03	10.65	**0.002**
	IOD	1	1.63E-02	13.38	**0.001**
Site	HL	1	7.91E-04	0.96	0.331
	JL	1	2.17E-04	0.33	0.568
	HW	1	2.09E-03	2.96	0.090
	IOD	1	6.62E-04	0.54	0.464
Error	HL	70	8.27E-04		
	JL	70	6.60E-04		
	HW	70	7.08E-04		
	IOD	70	1.33E-03		

Table 2.17 (continued)

(C) At 240 days

Source of variation	DV	df	MS	F	p
Covariate (SVL)	HL	1	1.91E-02	28.07	**<0.001**
	JL	1	1.17E-02	16.68	**<0.001**
	HW	1	1.58E-02	14.20	**<0.001**
	IOD	1	6.66E-03	5.51	**0.023**
Diet	HL	2	6.09E-05	0.09	0.915
	JL	2	3.16E-04	0.45	0.641
	HW	2	5.75E-04	0.52	0.600
	IOD	2	2.05E-03	1.69	0.194
Sex	HL	1	1.00E-02	14.73	**<0.001**
	JL	1	9.81E-03	13.98	**<0.001**
	HW	1	6.62E-04	0.60	0.444
	IOD	1	1.51E-03	1.25	0.270
Site	HL	1	1.69E-04	0.25	0.620
	JL	1	9.56E-05	0.14	0.714
	HW	1	1.09E-04	0.10	0.756
	IOD	1	3.09E-05	0.03	0.874
Error	HL	49			
	JL	49			
	HW	49			
	IOD	49			

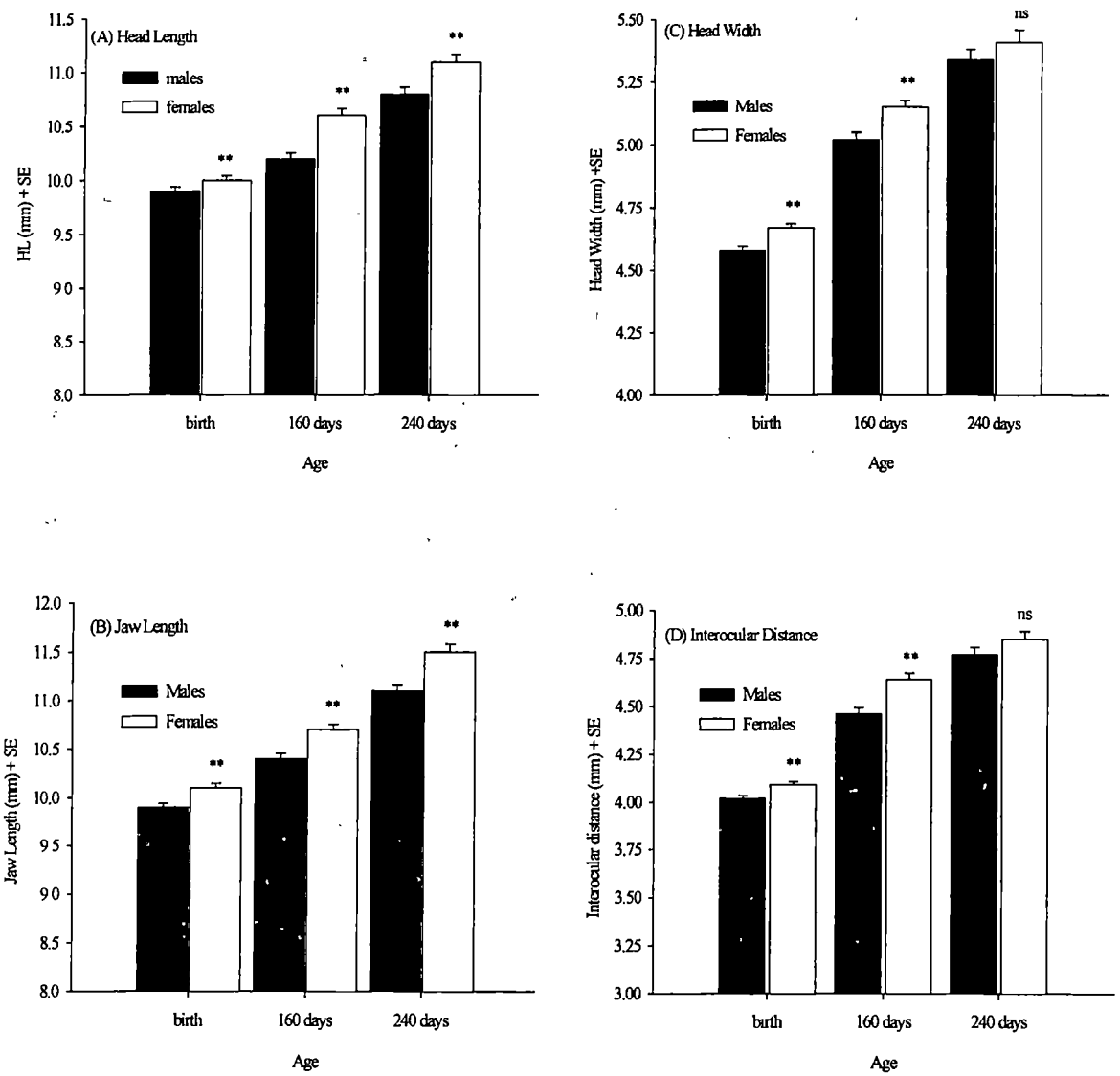


Figure 2.9: Sex differences in head length, HL (A), jaw length, JL (B), head width, HW (C), and inter-ocular distance, IOD (D) in garter snakes at birth, 160 days, and 240 days. Sample sizes were as follows: **Birth**: males, n = 70; females, n = 75. **160 days**: males, n = 37; females, n = 39. **240 days**: males, n = 27; females, n = 29. * = $p < 0.05$, ** = $p < 0.001$, ns = non-significant differences among sexes at each age.

snakes, possibly in response to dietary variation (Forsman & Lindell, 1991; Madsen & Shine, 1993). The close proximity of the two sites that I studied renders it unlikely that genetic modifications account for any geographic variation of body size. However, confirming that phenotypic plasticity accounts for size variation in the adult snakes among sites is complicated by certain factors. For example, one limitation of my study concerns survivorship of the snakes at each site. Because the precise ages of the adult snakes that I studied could not be determined, it remains unknown whether differences in survivorship among sexes and sites, rather than plasticity, accounts for the differences that I found. This is a limitation for many field studies of this type, especially those that do not measure growth patterns throughout the life span. The following discussion of my field work will assume survivorship to be equal among sexes and sites, and hopefully future work can address this potential bias.

Sexual size dimorphism in wild-caught garter snakes

Female wild-caught *T. sirtalis* that fed on amphibian-rich diets at Miller's marsh had significantly greater SVLs than females that fed primarily on earthworms at McCafferty farm. Females from Miller's marsh were also significantly heavier. Controlling for SVL, the masses of gravid females did not differ among sites. Interpreting differences in body mass of wild-caught snakes is complicated by several factors, including the amount of food possibly consumed prior to measurement (although any stomach contents were removed before weighing), stage of pregnancy, and the relatively small sample of males and non-gravid females compared in this study. However, SVL serves as a better measure of growth plasticity because it is less sensitive to fluctuations due to recent feeding history than body mass.

There are at least two dietary correlates of head-size variation in snakes: feeding frequency and prey size. In my study, site was a significant overall factor influencing head size in adult snakes. However, IOD was the only measure in which the snakes at the two sites differed significantly, with the snakes from Miller's marsh having greater IODs than the snakes from McCafferty farm. It is difficult to determine whether or not site differences in IOD are due to dietary differences. Researchers examining diet-induced morphological plasticity typically measure HL and JL, but not IOD or HW (Forsman, 1996a; Forsman & Lindell, 1991). One exception is a laboratory study by Queral-Regil & King (1998) who included the IOD measure in their study of water snake (*Nerodia sipedon*) head size in relation to prey size and quantity. Queral-Regil and King (1998) found that water snakes feeding on large prey had greater JLs than snakes feeding on small prey, but IOD was not affected by differences in prey size or amount. Forsman and Lindell (1991) found that adders (*Vipera berus*) that fed frequently on large-bodied prey had significantly greater HLs than adders feeding less frequently on small-bodied prey, but IOD was not reported in their study. Thus, there are no studies to my knowledge that indicate that dietary differences correlate with significant differences in IOD. Researchers looking at similar topics could measure IOD to determine whether this is a width measurement that is sensitive to diet-induced change. The non-significant differences in HL and JL that I found may be explained by the effects of gene flow, which may prevent the divergence of head size plasticity between sites, at least along length dimensions. Alternatively, the body sizes of the amphibians consumed by the snakes at Miller's marsh may not be large enough, in comparison to worms, to induce significant differences in HL and JL. The snakes from Miller's marsh appear to eat more frequently than the snakes

from Miller's marsh, but differences in feeding frequency also appear to have no affect on HL or JL.

The greater relative head size of females versus males has been previously reported for *T. sirtalis* (Shine & Crews, 1988). I found sex differences in relative head sizes in several analyses, with females having larger relative head sizes than males. My comparison using all wild-caught garter snakes revealed significant sex differences in all four of the head measurements taken. However, when I restricted my analyses to adult snakes, only HL and JL differed among sexes. The proximate cause of sexually dimorphic head sizes is most likely due to the growth inhibitory effects of androgens on male garter snakes (Crews at al., 1985; Shine and Crews, 1988). Several ecological and evolutionary explanations for sexual size dimorphism of body and head sizes in snakes have been proposed (Shine, 1993, 1994). One explanation for body size differences concerns sexual selection. Larger males are expected when there is combat among males competing for mates. This has been confirmed for some species of snakes (see Schuett & Gillingham, 1989), but does not apply to garter snakes since male-male combat does not occur. Fitch (1981) proposed that female snakes are larger than males among viviparous species, but not for oviparous species. However, this hypothesis was rejected by in a review by Shine (1993) who did not find this trend. Food resource partitioning among the sexes is another possibility (Shine, 1991). Female *T. sirtalis* in the wild are known to consume larger prey than males (Fitch, 1982; White & Kolb, 1974). In my study, the significant site by sex interaction in SVL was due to the equal SVLs of males from both sites, and the much greater SVLs of the females from Miller's marsh compared to females from McCafferty farm. This finding could be explained by food resource partitioning

between sexes at Miller's marsh, if males at both sites primarily consumed worms. However, amphibians were found in the stomachs of males at Miller's marsh (see Table 2.2). Further exploration including a larger sample size, data on prey sizes, and with males from both sites of known ages, is needed.

Sexual size dimorphism and litter differences in neonatal garter snake morphology

Relatively few authors have investigated the ontogenetic origins and processes that give rise to adult sexual size dimorphism (but see King, 1997; Shine & Crews, 1988). Sexual size dimorphism of neonatal snakes is fairly widespread and has previously been reported for *T. sirtalis* (see King, et al., 1999; Shine, 1993; but see Arnold & Peterson 1989). King et al. point out that the prenatal origins of sex differences in body and head size indicate that the fitness consequences of sexual dimorphism may exist prior to adulthood. At birth, males in my study had greater SVLs than females, and, less surprisingly, greater TLs. Male biased sexual dimorphism of TL persists into adulthood, but adult female SVLs are typically greater than male SVLs. Female neonates were significantly heavier than males, which may be due to a greater maternal investment of nutrients in female offspring. Further research could examine whether there are sex differences in survival among neonatal *T. sirtalis*, and whether any differences relate to birth weight.

It remains unclear whether sexual size dimorphism of adult garter snake head sizes has fitness consequences, or even that head size dimorphism correlates consistently with ecological factors (e.g., food resource partitioning). The presence of sexual size dimorphism among neonatal *T. sirtalis* suggests that its ecological significance can be found in early development. However, the statistical difference in neonatal head sizes

does not necessarily indicate a biologically relevant consequence (King et al., 1999). Sex differences in neonatal diets are difficult to determine at present given the paucity of field data available. Lind and Welsh (1994) compared the foraging behavior and diets of neonatal and adult *Thamnophis atratus*. Juveniles and neonates in Lind and Welshs' study foraged along stream margins and consumed smaller prey than adult snakes foraging in a wider variety of habitats. In nature, neonatal garter snakes of both sexes probably consume very small prey (e.g., worms, larval amphibians) simply because they are constrained by their small heads. At present, field data on the diets of very young snakes of both sexes are needed, as well as detailed laboratory studies comparing swallowing performance in male and females snakes with head size dimorphism.

Litter effects

King et al. (1999) emphasized the importance of including litter as a factor in tests for sex and site effects on neonatal morphology in snakes, and demonstrated that sex effects would have gone undetected had family membership been ignored. The litter effects found for all morphological measurements in my dissertation support the case made by King et al. There may be several sources explaining litter variation, which were not determined in my study or by King et al. Maternal effects on neonatal morphology (e.g., scalation, growth) are known to be relatively great (King et al., in review). However, I found that maternal SVL did not predict neonatal SVL or mass, but a larger sample may yield significant relationships, as all slopes were positive. Genotypic effects may also explain litter differences. The values of some morphological measures are heritable, as demonstrated by King (1997) for JL and TL in *Storeria dekayi*.

The sex and litter effects that I found, and those of King et al. (1999) are not consistent with all studies of neonatal morphology in *T. sirtalis*. Arnold and Peterson (1989) report no sexual size dimorphism or significant litter differences in body size, HL, HW, and eye diameter among neonatal *T. sirtalis*. The discrepancies among the studies may reflect geographic variation in neonatal morphology, but differences in sample sizes or maternal effects may be a contributing factor. The sample size of Arnold and Peterson (1989) was low (n = 65 individuals, 9 litters) in comparison to mine (n = 286 individuals, 33 litters), and King et al.'s (n = 407 individuals, 27 litters). Regardless, litter differences in neonatal morphology may have important ecological and evolutionary implications. Further studies on the contributions of maternal, genetic, and early environmental influences on morphology and growth are needed (e.g., King et al., in review).

Diet and growth

Laboratory reared garter snakes grew significantly in SVL and mass between birth and 240 days, but diet-induced size differences were not detected until the snakes reached 240 days. The latter finding suggests that feeding on a mixed diet promotes rapid growth later in life (160-240 days), in comparison to feeding on worms or fish alone. The significant increase in body size in the mixed diet group that I found differs from the results of previous research on *T. sirtalis* (Burghardt & Krause, 1999; Lyman-Henley & Burghardt, 1995; Scudder-Davis & Burghardt, 1987). Burghardt and Krause (1999) report significantly greater increases in SVL at 80 days in snakes reared on worm or mixed diets over snakes feeding on fish alone. The neonates tested in that study were born to mothers collected at McCafferty farm. My failure to replicate this finding could be due to several factors.

The cause of the discrepant findings between my study and Burghardt and Krause's (1999) is probably not due to differences in laboratory conditions, as they were nearly identical in both studies. The discrepancy may be due to the different species of fish used. In my study, *Pimephales promelas* were used for the F group, whereas Burghardt & Krause (1999) used *Gambusia affinis*. Thus, the differences in the F group growth rates between the two studies may be explained by the dietary quality of the fish used. However, this is unlikely and alternative explanations may be more helpful. Proximate factors such as maternal diet (Ford & Seigel, 1989) and temperature (Arnold & Peterson, 1989) can affect neonatal morphology and juvenile growth respectively. Arnold and Peterson (1989) found that juvenile *T. sirtalis* reared in warmer temperatures grew more rapidly than snakes reared in colder temperatures. The authors speculated that prenatal temperature could influence snake growth rates. Thus, with regard to my study and Burghardt and Krause's (1999), the maternal diets of the mothers used in the two studies may have differed prior to capture, possibly due to differences in prey abundance between the two years (1996 vs. 1998). Arnold and Peterson's (1989) suggestion that prenatal temperature affects postnatal growth rates also may explain the discrepancy between the two studies.

Sex did not have a significant effect on growth rate, but has been shown to be a factor in previous reports of *T. sirtalis* growth (Crews, at al., 1985). The discrepancy between the findings of Crews et al. and the present study may be due to methodological differences, or because of inherent differences between the populations sampled. Another possible explanation is the relatively small sample size used in the present study. Significant effects of food size and feeding frequency on head size have been reported for

European adders, *Vipera berus* (Forsman & Lindell, 1991), though head size variation may reflect microevolutionary change rather than phenotypic plasticity (Forsman, 1996a). The non-significant effect of diet on relative head sizes found in my study may be related to the prey species used, or to the feeding regimen. The snakes were fed an equal number of times. Increasing the frequency of feeding through the first 8 months may have resulted in a significant effect of diet on relative head size (e.g., Queral-Regil & King, 1998). In addition, the prey species used may not have differed substantially enough in morphology (e.g., size, hardness) to produce such change. Data on captive American alligators has shown that captive rearing can result in reduced length of the rostrum and snout strength (Meers, in prep.). A similar explanation could account for the non-significant differences in head sizes among the diet groups in my study.

Bone structures can be modified in size and shape by environmental factors such as nutrition and use, and the degree to which remodeling occurs varies with the amount of mechanical strain placed upon bones (Lanyon & Rubin, 1985). The bone morphology of the head can vary due to differences in jaw muscle activity associated with diet (Robinson & Wilson, 1995; Queral-Regil & King, 1998; Walls, Belanger, & Blaustein, 1993). The leg bones of Anolis lizards (*Anolis sagrei*) can undergo changes in size that facilitate efficient movement on different types of substrate (Losos et al., 2000). However, the degree to which the bone size can be remodeled due to muscular activity and strain may be constrained by microevolutionary factors, which may explain why diet did not affect the head sizes of neonates reared on different diets in my study, and the lack of site differences in JL and HL in adults from the two sites (see also Forsman, 1996a).

CHAPTER 3

EXPERIENTIAL MODIFICATION OF CHEMICALLY MEDIATED RESPONSES

Introduction

Thamnophis sirtalis attacks and shows increased tongue-flick rates toward a wide variety of prey chemicals prior to feeding experience (Burghardt, 1969). However, feeding experience interacts with the genetic control of garter snake chemosensory responses (Burghardt, 1993; Burghardt et al., 2000). For example, Fuchs and Burghardt (1971) found that genetically controlled chemical preferences for prey could be modified with feeding experience in very young garter snakes. Similarly, Lyman-Henley and Burghardt (1995) reared neonatal *Thamnophis butleri*, a worm specialist, and *T. sirtalis* on either fish or worm diets for 157 days and tested their chemical prey preferences to surface extracts of both stimuli prior to and following this period. *Thamnophis butleri* responded strongly to worm extract, but the group reared on fish responded equally to worm and fish extracts. *Thamnophis sirtalis* showed a modest increase in their chemosensory responses to familiar prey extracts. However, as Lyman-Henley and Burghardt (1995) pointed out, their propensity to attack both fish and worm extracts is consistent with their generalist nature.

Exposure to prey chemical stimuli in the absence of feeding in neonatal snakes influences chemosensory responses and prey choice in the opposite fashion. For example, Burghardt (1992) exposed neonatal *T. sirtalis* to constant, ambient vomodors of either live fish or worms for several days, and subsequently measured the snakes' tongue-flick rates and attack latencies to surface extracts of both stimuli. The snakes in both

experimental groups showed preferences for the prey chemicals that they had not previously experienced. Furthermore, this chemical experience significantly affected prey choice; in simultaneous prey choice tests the snakes were less likely to eat the prey associated with the odor presented. Overall, however, the snakes were more likely to eat worms, which is typical of neonatal and adult *T. sirtalis* in the wild (Burghardt, 1992).

Long-term developmental studies of snake chemosensory responses have revealed important findings. Mushinsky and Lotz (1980) found that water snakes (*Nerodia erythrogaster*) shifted their chemosensory responses from one prey type to another, not because of feeding experience, but when body sizes increased to levels that would accommodate large bodied prey. Genetically controlled ontogenetic shifts in prey chemical preferences, like those found in *Nerodia erythrogaster* and *Regina alleni* (see Waters, 2000), are not likely to occur in *T. sirtalis*, as neonates of the latter species attack prey chemicals of both large and small-bodied prey. However, prey chemical preference shifts have been reported in *T. sirtalis* when diet was modified. Fuchs and Burghardt (1971) found this for very young laboratory born *T. sirtalis*. Greenwell et al. (1984) reported worm chemical preferences by neonatal *T. sirtalis* born to mothers from High Island (Charlevoix County, MI), which is a part of the Beaver Archipelago. However, no selective preferences for fish, amphibian, and worm extracts were shown for adults collected at High Island. Although the relative contributions of maturation and experience were not determined by Greenwell et al., their study demonstrates plasticity of chemosensory responses in *T. sirtalis*.

In my experiment, chemosensory responses to fish and worm extracts were tested intermittently until the snakes reached 242 days. My primary interest was in determining

how initial diet and diet switching affect the snakes' chemosensory responses through their first 8 months. Fuchs and Burghardt (1971) found that very young garter snakes responded more strongly to extracts of prey that they had recently eaten than to prey they had not eaten recently, or at all. This may have adaptive significance for neonatal garter snakes in the wild as they acquire foraging skills. Increased sensitivity to familiar prey may facilitate the process of locating food, which may be especially important to neonatal garter snakes that forage in relatively restricted habitats. I tested whether garter snakes show elevated responses to recently ingested prey at a later age. However, based on the findings of Arnold (1978) and Burghardt, Konigsberg, and Layne (20000), elevated responses should only occur toward fish extract following experience with feeding on fish. Feeding on worms or amphibians, which are staple prey items in garter snake diets, is less likely result in increased chemosensory responses to these prey items. Thus, garter snakes reared initially on fish or that switch to eating fish following an initial diet of worms should show increased responses to fish extracts. I also sought to determine how site, sex, and litter affect the snakes' chemosensory responses prior to and following feeding experience. If sex-based food resource partitioning occurs in garter snakes, the sex differences in chemosensory responses could be expected. Also, litter variation in common garter snake chemosensory responses has been reported (Burghardt, 1975; Burghardt, 1993; Lyman-Henley & Burghardt, 1995) and I tested this possibility in my study.

Method

Subjects and maintenance

Subjects were 78 neonatal *T. sirtalis* (42 males, 36 females) from 17 litters (M litter size = 4.6, range = 1-13) born to females collected at Miller's marsh (n = 8 litters) and McCafferty farm (n = 9 litters) in 1998 (see Appendix I for specific litters tested). Housing conditions were the same as those described for neonates in Chapter 2. These snakes were also used for prey handling tests (see Chapter 4, Experiment I).

Procedure

At birth, the snakes were divided into three feeding groups, Fish (F group, n = 22), Worm (W group, n = 29), and Mixed (FW group, n = 27). Diet designations were balanced as well as possible across sex, litter, and site. There were four testing periods, Chem1 (17-19 days of age) which was done prior to the snakes' first feeding, Chem2 (80-82 days), Chem3 (160-162 days), and Chem4 (240-242 days). The testing and feeding schedules for each diet group are summarized in Table 3.1. At each testing period, the snakes' responses to three mildly diluted extracts each of fish (*Pimephales promelas*), worm (*Lumbricus rubellus*), and to distilled water (control) were recorded (see Burghardt, 1969 for extract preparation methods). Each stimulus presentation was separated by a 15 to 20 minute interval, with the snakes tested on either fish or worm extract. After 1 hour, the tests were repeated, with each snake exposed to the opposite stimulus of that used in the first set of trials. Water was always presented first, followed by increasing concentrations (1/3, 2/3, and full strength) of prey extract, for a total of eight stimulus presentations per day for each snake. The order of prey stimulus presentation was randomized, with an equal number of snakes first exposed to fish, and

Table 3.1: Chemical extract testing schedule for the three diet groups.

		Diet group		
		Fish (F)	Worm (W)	Mixed (FW)
Test Period	*Age (days)	Diet preceding test	Diet preceding test	Diet preceding test
Chem1	17&19	Naive (n = 16)	Naive (n = 20)	Naive (n = 18)
Chem2	80&82	Fish (n = 11)	Worm (n = 15)	Fish & Worm (n = 8)
Chem3	160&162	Fish (n = 13)	Worm (n = 16)	Fish & Worm (n = 15)
Chem4	240&242	Worm (n = 10)	Fish (n = 15)	Fish & Worm (n = 11)

*Two blocks of trials were conducted at each test period, always within two days of each other. A total of eight stimulus presentations occurred at each block: 2 presentations of the control stimulus and 3 presentations of each prey extract (fish and worm).

an equal number first exposed to worm at each testing period. A second block of trials was conducted two days later using the same method described above, but with the order of prey stimulus presentations reversed. Room temperature was kept at around 23° C and humidity at 55%. Fifty-four of the 78 snakes were tested for responses to prey extracts at Chem1 (F group, n = 16; W group, n = 20; FW group, n = 18). The remaining 24 snakes served as controls for another study, and were added to the sample used in this experiment at a later time (see below for explanation). These 24 snakes were tested using the same method described above, but with distilled water used as the stimulus for each trial. Following Chem1 (20 days of age), all 78 snakes were offered their first meal corresponding to their designated diet group. The first meal for the FW group was randomly determined, with an equal number of snakes receiving fish or worm first, and the opposite prey item was offered two days afterward. These initial feedings were videotaped as a part of the prey handling experiment (see Chapter 4, Experiment I). The snakes were then fed once weekly until the next testing period. To minimize hunger effects, feeding was stopped 4-7 days prior to each chemical extract testing period. Chemical extract tests 1, 2 and 3 were conducted to test for long term effects of diet on chemosensory responses. Following Chem3 (160-162 days), diets for the F and W groups were reversed, and Chem4 was completed at 242 days to determine whether food switching affected chemosensory responses at a relatively late age. Due to subject mortality, the sample size diminished from 54 snakes to 21 snakes between Chem1 and Chem3. Therefore, 23 of the 24 littermates that served as controls at Chem1 were added to the sample for Chem3 and Chem4.

Statistical analyses

Tongue-flick/attack scores (TFAS) were calculated for responses to each stimulus at each testing period. The scoring method, adopted from Cooper and Burghardt (1990), used the formula $TFAS(R) = TF_{max} + (TL - latency)$, where R specifies that the formula accounts for repeated testing of individual snakes. TF_{max} is the maximum number of tongue-flicks recorded toward a single stimulus within a repeated series of tests by the individual snakes. TL is the length of the trial in seconds (always 30 s in this case), and $latency_i$ is the attack latency (in seconds) at each trial. Comparisons of TFASs between testing periods (Chem1 - Chem4) were done using the averaged values of the first and second blocks of trials within each test period (see Table 3.1).

Responses to fish, worm, and control stimuli were compared at each testing period using repeated measures ANOVA. Then, a MANOVA was used to test for the effects of diet, litter, sex, and site on the snakes' TFASs to fish and worm extracts. Litter was treated as a random factor and was nested within site. Stimulus concentration effects were tested using separate repeated measures ANOVAs at each test period. To determine whether diet had a significant effect on TFASs across testing periods, two separate MANOVA for repeated measures tests (O'Brien & Kaiser, 1985) were performed. The first tested for differences in responses to fish and worm extract between Chem1 and Chem2 and the second tested for differences to both stimuli between Chem3 and Chem4. The first MANOVA determined whether initial diet affects chemosensory responses to either prey stimulus. The second MANOVA tested for the effects of diet switching on chemosensory responses to either stimulus. The differences in TFASs to each stimulus were calculated separately (Chem2-Chem1, and Chem4-Chem3) and were treated as

dependent variables. The repeated measures MANOVAs were computed separately because subjects were added at Chem3 to compensate for mortality.

Another technique for analyzing this type of data set is to test for changes in relative preference scores across testing periods (see Burghardt et al., 2000). First, relative preference scores were obtained by subtracting worm scores from fish scores at each test period. Then, changes in relative preference scores were obtained by subtracting the relative preference scores at Chem1 from the relative preference scores at Chem2. These calculations were also used to compare changes in relative preference scores between Chem3 and Chem4. Two separate univariate F-tests were used to compare changes in relative preference scores between Chem1 and Chem2, and between Chem3 and Chem4.

All data were normalized using natural log (+1) transformations. Repeatabilities within each test period and across all test periods were calculated separately for each stimulus using intraclass correlation coefficients. Coefficients ranged from 0.19 to 0.83, and all were significant (see Table 3.2).

Results

Stimulus concentration did not have any effect on the chemical sensory responses to either fish or worm at any of the test periods. Thus, the TFASs for the three concentrations within each testing period were averaged (6 presentations/stimulus) for each prey item.

Chemosensory responses prior to feeding

At Chem1, the snakes showed higher responses to fish and worm extracts compared to the control stimulus ($F = 32.10$, $df = 2, 106$, $p < 0.001$), and there was a

Table 3.2: Repeatability of chemosensory responses to distilled water, fish, and worm extracts within and across all test periods. Significant p-values are boldfaced.

	Chem1	Chem2	Chem3	Chem4	Total
Stimulus	R, p	R, p	R, p	R, p	R, p
Control	0.274	0.476	0.20	0.431	0.188
	<0.001	**<0.001**	**0.001**	**<0.001**	**<0.001**
Fish	0.597	0.808	0.58	0.688	0.416
	<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**
Worm	0.575	0.52	0.585	0.828	0.282
	<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**

Note: R = intraclass correlation coefficient.

slight but significant overall preference for fish over worm extract for all snakes combined ($t = 2.15$, $df = 53$, $p = 0.036$, Figure 3.1). At Chem1, there was no overall effect for diet group ($\lambda = 0.752$, $F = 1.98$, $df = 2, 12$, $p = 0.181$), indicating that none of the three diet groups showed significant preferences for either prey extract prior to feeding. No overall effects were found for sex at Chem1 ($\lambda = 0.899$, $F = 0.68$, $df = 2, 12$, $p = 0.527$), or for site ($\lambda = 0.892$, $F = 0.73$, $df = 2, 12$, $p = 0.503$). A marginally significant overall effect was found for litter at Chem1 ($\lambda = 0.20$, $F = 1.75$, $df = 26, 36$, $p = 0.06$). Univariate F-tests revealed significant litter effects for responses to both fish and worm stimuli, but no effects for sex, site, or diet (Figure 3.2A-F) in response to fish or worm stimuli (Table 3.3).

Chemosensory responses after feeding experience

Significant stimulus effects were found at Chem2 ($F = 35.53$, $df = 2, 62$, $p < 0.001$), indicating that responses to prey extracts were greater than responses to the control stimulus (Figure 3.2A-F). Sex and site effects were not significant and were removed from the MANOVA. Diet had a marginally significant overall effect on the development of chemosensory responses to prey stimuli ($\lambda = 0.317$, $F = 2.72$, $df = 4, 14$, $p = 0.073$). However, a significant effect was found for responses to fish extract, but not to worm extract (Table 3.4). The diet effect was primarily due to the FW group's greater ($p = 0.044$) relative increase in response strength to fish over the W group. Litter did not have a significant effect on changes in chemosensory responses between Chem1 and Chem2.

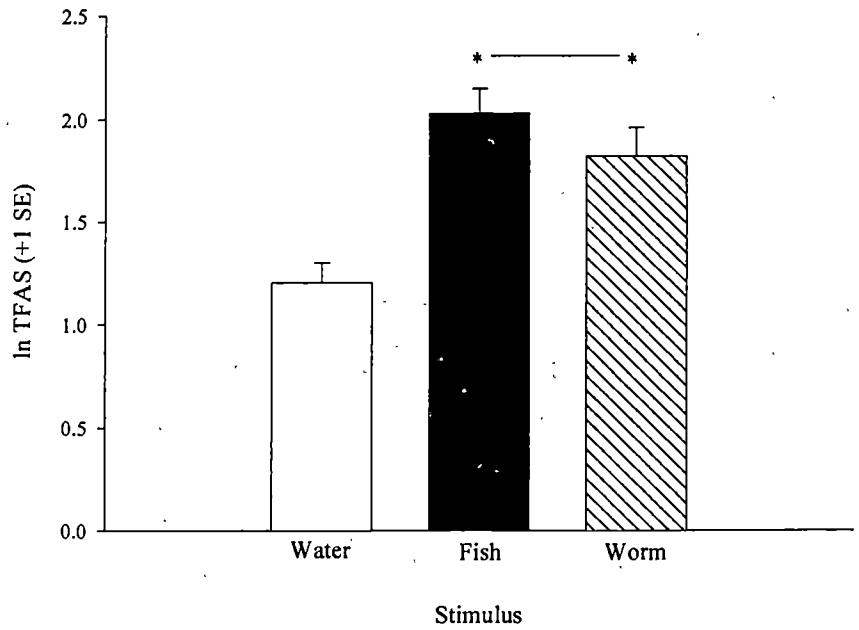


Figure 3.1: Initial (Chem1) chemosensory responses to fish, worm, and control stimuli by 54 neonatal garter snakes. * = $p < 0.05$ significant difference in TFASs to fish and worm extracts.

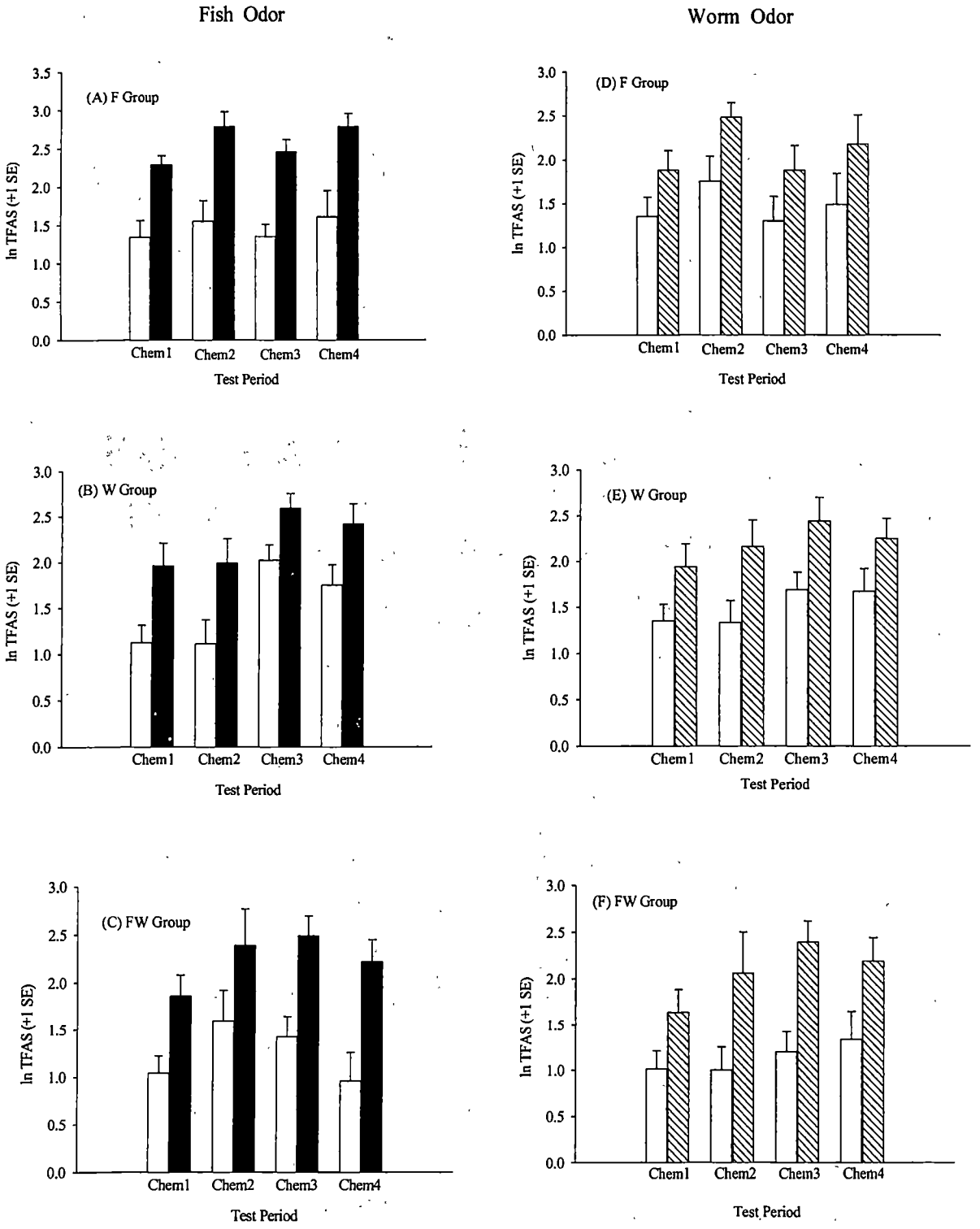


Figure 3.2: Responses to fish (left panel) and worm (right panel) extracts at Chem1-Chem4 by snakes in the F, W, and FW groups during. Diets were switched for the F and W groups following Chem3. Control = open bars, Fish = dark bars, Worm = shaded bars.

Table 3.3: Results from the MANOVA testing for site, sex, diet, and litter effects on neonatal chemosensory responses to fish and worm extracts prior to feeding experience (Chem1).

Source	DV	df	Hypothesis MS	Error MS	F	p
Site	Worm	1, 13	0.527	1.76	0.30	0.593
	Fish	1, 13	1.27	1.25	1.02	0.331
Sex	Worm	1, 13	1.56	1.76	0.89	0.364
	Fish	1, 13	0.29	1.25	0.23	0.640
Diet	Worm	1, 13	0.014	1.76	0.01	0.931
	Fish	1, 13	0.976	1.25	0.78	0.393
Litter within Site	Worm	13, 19	1.76	0.54	3.24	**0.01**
	Fish	13, 19	1.25	0.52	2.41	**0.04**

Table 3.4: Results of repeated measures MANOVA testing for diet and litter effects on chemosensory responses to fish and worm extracts after feeding experience (Chem1 - Chem2) by garter snakes reared on three different diets.

Source	DV	df	MS	F	p
Diet	Worm	2, 8	1.25	2.07	0.188
	Fish	2, 8	4.12	6.18	**0.024**
Litter	Worm	14, 8	0.97	1.62	0.251
	Fish	14, 8	0.49	0.74	0.703
Diet*Litter	Worm	9, 8	1.24	2.06	0.161
	Fish	9, 8	1.18	1.77	0.217
Error	Worm	8	0.60		
	Fish	8	0.67		

My analyses for changes in relative preference scores resulted in the same general findings reported above. A significant effect for diet was found ($F = 5.59$, $df = 2, 31$, $p = 0.008$), indicating that feeding experience influenced the snakes' responses to prey extracts. As with the repeated measures MANOVA reported above, multiple comparisons showed a significantly ($p = 0.006$) greater increase in response to fish extract by the FW group over the W group between Chem1 and Chem2, but no difference between the F and W groups.

Effects of diet switching

Overall, the snakes responded more to prey stimuli than to the control stimulus at Chem3 ($F = 30.85$, $df = 2, 80$, $p < 0.001$), and at Chem4 ($F = 42.36$, $df = 2, 62$, $p < 0.001$). Due to subject mortality, litter sizes diminished between Chem3 and Chem4. Thus, litter was removed from the MANOVA testing for effects of diet switching. With litter, site, and sex removed, diet was the only factor used in the MANOVA. In this test, diet did not have a significant overall effect on chemosensory responses to prey stimuli ($\lambda = 0.798$, $F = 1.73$, $df = 4, 58$, $p = 0.155$, Table 3.5). Thus, diet switching at 160 days does not result in additional chemosensory response changes by 240 days of age. Removing the FW group from the analysis did not change these results.

Analyses on relative preference scores did not change the results for the diet-switching phase of this experiment. No significant effect was found for diet ($F = 0.65$, $df = 2, 30$, $p = 0.530$) and none of the diet groups differed significantly from one another.

Table 3.5: Results of repeated measures MANOVA testing for diet effects on chemosensory responses to fish and worm extracts after diet switching (Chem3 - Chem4) by garter snakes reared on three different diets.

Source	DV	df	MS	F	p
Diet	Worm	2	2.21	2.70	0.084
	Fish	2	1.38	2.00	0.152
Error	Worm	30	0.82		
	Fish	30	0.69		

Discussion

Chemosensory responses to prey extracts and the control stimulus were repeatable, demonstrating that chemosensory responses to each prey type are robust through the first 242 days. Overall response levels to all stimuli, including controls generally increased between 17 and 242 days. The increased levels of responsivity to stimuli may be based on the physical maturation of the snakes, or due to feeding experience. In addition, conditioning (e.g., sensitization) to the swab test procedure may have occurred. However, the MANOVA for repeated measures that I used analyzed linear changes in responses to *each* prey stimulus across two pairs of test periods (Chem1 vs. Chem2, and Chem3 vs. Chem4). Thus, if sensitization effects explain the diet effect that I found for the FW group, then significant effects should have appeared for both fish and worm stimuli.

Prey dependent changes in chemosensory responses

Snakes feeding on the mixed diet increased their chemosensory responses to fish extract after feeding experience. However, the chemosensory responses of the F and W groups were not significantly affected by diet. The increased response to fish extract by the FW group may indicate a prey preference that could not be expressed by the F or W groups because of their dietary restrictions. The results from my tests on the effects of diet on chemosensory responses differ from previously reported data from Beaver Island *T. sirtalis* (Burghardt et al., 2000). Burghardt et al. found that neonatal *T. sirtalis* from Beaver Island significantly increased their chemosensory responses to fish following 12 meals consisting exclusively of fish. Arnold (1978) also found that feeding experience with fish resulted in increased response levels to fish extract by *T. sirtalis*. The snakes

reared exclusively on fish diets in my dissertation increased their responses to fish extract with feeding experience but the change was not significant.

Prior to feeding experience, the snakes in the F group responded more strongly to fish extract than the W and FW groups. The slight, initial bias by the snakes toward fish extract suggests a genetically controlled preference and may explain why the F group's mean response to fish extract did not significantly increase. This result is peculiar due to the fact that neonatal *T. sirtalis* primarily feed on worms, and fish seem to be fed upon more opportunistically than other prey classes, such as amphibians (Gregory & Nelson, 1991; Nelson & Gregory, 2000). Individuals and litters of *T. sirtalis* are known to have stable chemoreceptive response biases toward particular prey classes (Burghardt, 1975; Lyman-Henley & Burghardt, 1995). Also, prior to feeding experience, I found litter effects for responses to both fish and worm extracts. The fish bias was taken from the averaged TFASs of 17 litters born to females collected during the 1998 field season. Burghardt et al. (2000) report no significant preference for fish or worms by neonates born to 17 females collected at Jordan River, a site adjacent to McCafferty farm where the snakes primarily consume worms. Ideally, in future studies of this type, initial chemosensory response profiles could be examined prior to determining the snakes' initial diets, and diet assignments could be made such that the average responses to each stimulus are balanced between diet groups. However, this would probably compromise controls for sex, litter, and population (see Burghardt & Schwartz, 1999 for discussion on methodological issues).

One similarity between the studies of Arnold (1978), Burghardt et al. (2000), and mine is the increase in chemosensory responses to fish extract by *T. sirtalis* with fish in

their diets. Arnold (1978) also reared garter snakes on frogs and worms, but their chemosensory responses to these two prey species were not affected by feeding experience. Burghardt et al. found that a change in relative preference for fish extract, but not worm extract, was heritable. Responses to fish extract by garter snakes may be more modifiable with feeding experience; the aquatic foraging abilities of *T. sirtalis* are fairly unspecialized. Across its entire geographic range, *T. sirtalis* appears to be more likely to feed on amphibians and worms than on fish. Increased sensitivity toward atypical prey may facilitate the invasion of new feeding niches. Further work could explore whether the chemosensory responses to other less frequently ingested prey increase with feeding experience on these prey.

Age differences in diet-induced chemosensory response changes

Diet-induced modifications of chemosensory responses may have advantages, such as the formation of "search images", as proposed by Fuchs and Burghardt (1971). However, if search images develop, as they appear to for some snakes (see also Burghardt, 1990), they may be subject to extensive modification as snakes age or encounter other prey types. Also, search images are known to be short lived (see Tinbergen, 1960, cited in Shettleworth, 1998). Repeated extract testing immediately following initial feedings or diet switching may be needed to detect search images, as in Fuchs and Burghardt (1971).

Overall, the results from studies of diet effects on garter snake chemosensory responses are somewhat mixed. One explanation may be geographic variation in garter snake chemosensory responses (see Arnold, 1992; Burghardt & Schwartz, 1999). Another explanation may involve methodological differences among studies. For

example, Arnold (1978) and Fuchs and Burghardt (1971) studied the effects of diet on very young garter snakes (10 - 53 days, and 6 - 41 days, respectively). Using a single diet design to examine quantitative genetics of chemosensory responses, Burghardt et al. (2000) studied garter snakes from seven through 70 days of age. Lyman-Henley and Burghardt (1995) tracked diet effects on chemosensory responses in garter snakes through 159 days. I tested my snakes from 17 through 242 days of age. Thus, in addition to geographic variation, differences in age may account for differences in results, especially in studies that included a diet-switching phase (e.g., present study; Fuchs & Burghardt, 1971). Additional research could address this issue using a very large number of litters from a single population, and manipulating the timing when diet switches are imposed, with littermates spread equally across treatments. Apparently, diet-switching affects the chemosensory responses of very young snakes (e.g., Fuchs & Burghardt, 1971), but the effects were not apparent at 240 days in my study. Neonatal garter snakes may develop increased chemical sensitivity to prey that are in their immediate environment because the types of habitats they forage in, and their wandering ranges, are quite limited in comparison to older snakes (Lind & Welsh, 1994). When garter snakes become more proficient foragers, their chemosensory responses may be less likely to change as a result of feeding experience because they are less constrained by prey size, limited use of habitat, and relatively short wandering ranges.

CHAPTER 4

THE ONTOGENY OF FEEDING SKILLS

Introduction

The types of environments in which animals forage, and the variability of prey abundance across both spatial and temporal scales, are known to influence the degree to which foraging efficiency increases with feeding experience (Day and McPhail, 1996; Ehlinger, 1989; Krebs and Inman, 1994). The common garter snake (*Thamnophis sirtalis*), the most geographically widespread species of snake in North America, feeds on a diversity of prey species that vary in spatial and temporal abundance (see Rossman, Ford, and Seigel, 1996). The progeny of predatory and habitat generalists, such as neonatal *T. sirtalis*, may be born into highly fluctuating environments where learning is vital to foraging success; their survival may depend upon their abilities to detect, capture, and consume prey. Empirical studies have demonstrated a close relationship between juvenile survival, reproductive success, and foraging proficiency (Sih, 1993). For example, birds that are slow to acquire foraging skills are known to delay breeding, starve, or suffer increased predation risk (see Yoerg, 1994). Even highly precocial species may require feeding experience in order to forage efficiently (Burghardt & Krause, 1999; Croy & Hughes, 1991a,b; Day & McPhail, 1996; Mori, 1996; Savitsky & Burghardt, 2000).

Nevertheless, although plasticity, especially learning, may be beneficial, there are costs to relying on experience for developing foraging skills. These include increases in both predation risk and time and energy devoted to finding and consuming prey. These

costs may be most evident during early development, and have been reported in studies comparing specialist and generalist snake species. For example, neonates of generalist species are often inferior in foraging proficiency compared to morphologically and behaviorally specialized snakes (e.g., Drummond, 1983; Halloy & Burghardt, 1990; Mori, 1994, 1996). However, with experience predatory generalists can become nearly as proficient at consuming prey as specialist species. For example, Mori (1996) compared rodent handling in hatchling, yearling, and juvenile *Elaphe climacophora*, a rodent specialist, with *E. quadrivirgata*, a dietary generalist. With feeding experience, *E. quadrivirgata* were nearly equal in rodent handling ability to *E. climacophora*, whereas younger snakes were much less adept than *E. climacophora* at handling rodents.

Efficiently detecting, capturing, and consuming prey may minimize predation risks for garter snakes that forage in open fields, along water banks, and under water, where they themselves may be highly vulnerable to predators. The foraging repertoire of the common garter snake (*Thamnophis sirtalis*) shows a high degree of plasticity (Burghardt, 1993; Burghardt & Krause, 1999; Halloy & Burghardt, 1990). *T. sirtalis* is capable of detecting, subduing, and consuming a wide variety of prey species including annelids, fish, amphibians, mammals and birds. The rapid acquisition of feeding skills by *T. sirtalis* would aid in reducing the costs of being relatively unspecialized. Behavioral plasticity would facilitate the acquisition of feeding on both novel and species-typical prey. For example, *T. sirtalis* in most populations feed on earthworms and a variety of amphibian species, but will feed on fish opportunistically in the field and readily in captivity (Arnold, 1992; Carpenter, 1952; Gregory & Nelson, 1991; Nelson & Gregory, 2000). However, neonatal *T. sirtalis* are not very adept at handling fish in comparison to

Thamnophis melanogaster, an aquatic prey specialist (Halloy & Burghardt, 1990). With feeding experience, *T. sirtalis* is capable of consuming fish about as proficiently as *T. melanogaster*, thus benefiting from behavioral plasticity.

The degree to which learning contributes to the development of feeding skills may be condition dependent. That is, diet may determine how efficiently an individual will be in detecting, capturing, handling, and swallowing prey. For example, feeding on a mixed prey diet may impede predators from learning the most efficient techniques (Burghardt & Krause, 1999; Cunningham & Hughes, 1984). Thus, there are potential costs to feeding on a mixture of prey species. The costs, however, may not outweigh the benefits of feeding on several prey species. Furthermore, feeding on one type of prey may facilitate switching to another type, but interference effects can impede a predator's ability to undergo dietary shifts (Yeager, Burghardt, & Lyman-Henley, 1996). Interference effects can occur when a predator switches from a relatively easy prey to feed on, to a more difficult one. Retaining feeding skills may be critical as well, as costs may accrue if an individual forgets how to locate, capture, and consume prey (Hughes & Blight, 1999; Krebs & Inman, 1994; Shettleworth, 1998).

Previously, Burghardt and Krause (1999) tested three groups of neonatal *T. sirtalis* on their abilities to feed on fish, worms, or a mixed diet. Initially, all prey items took equally long to consume. However, after the three diet groups had 11 to 12 feedings on their respective diets, fish consumption times decreased significantly, and worm consumption times decreased for snakes feeding exclusively on worms. Also, the phases of predation were differentially affected by feeding experience. Fish and worm detection, as measured by prey approach times, decreased significantly after feeding experience by

snakes reared on pure diets, but not for snakes reared on mixed diets. Feeding on a mixed diet also appeared to interfere with the development of approaching, capturing, handling, and swallowing worms.

The findings reported in Burghardt and Krause (1999) lead to further questions about the ontogeny and plasticity of neonatal garter snake foraging behavior. This chapter of my dissertation extends the study of Burghardt and Krause (1999) in several directions by 1) including animals from two different, but nearby, sites where the animals live in different habitats and forage on different prey, and 2) observing foraging by adults freshly captured from each site. I conducted three experiments to address several questions on the ontogeny of foraging skills. The first question concerns the age at which young snakes reach asymptotic levels of prey consumption efficiency. The second question concerns the effects of switching from difficult to relatively easy to consume prey, and the reverse. It appears that feeding on fish, a prey item that is relatively difficult to consume, may facilitate switching to worms, which are easier to consume. Conversely, feeding on worms may interfere with switching to fish (Yeager et al., 1996). Will feeding on a mixed diet confer any foraging efficiency advantage over snakes reared on a single diet? The third question concerns the ability of garter snakes to retain acquired foraging skills after a period of having to depend on other prey, as may occur when snakes are confronted with periodic and extended fluctuations in prey abundance. Thus, the retention of feeding skills by garter snakes needs to be assessed. Forgetting how to handle prey has been reported in stickleback, *Spinachia spinachia* (Croy & Hughes, 1991a; Mackney & Hughes, 1995) and this possibility was tested for garter snakes in Experiment I. A fourth question concerns whether snakes from the two populations with different

food resources would either differ at birth in foraging proficiency on earthworms or fish, or in the plasticity of their learning. Experiment II examined whether feeding skills obtained after a lesser amount of feeding experience than in Experiment I are retained following diet switching.

Whether laboratory studies of neonatal garter snake foraging behavior (e.g., Burghardt & Krause, 1999; Halloy & Burghardt, 1990) apply to natural situations was tested in Experiment III. Adults captured from the two ecologically dissimilar sites were tested on their abilities to prey upon fish, frogs, and worms. Because their feeding experiences are restricted to worms, the adult garter snakes from McCafferty farm should have greater difficulty feeding upon large amphibians than snakes from Miller's marsh. Fish have not been recorded in the stomach samples from snakes at either site. I included fish prey in Experiment III to test whether snakes from Miller's marsh, owing to their more diverse diets, would show superior feeding skills on novel prey compared to snakes from McCafferty farm. Finally, I predicted equal worm consumption abilities by snakes from both sites.

Experiment I: Dietary Effects on Feeding Skills in Young Garter Snakes

In this experiment, I manipulated the diets and feeding schedules of young garter snakes such that the effects of initial feeding experience, prey switching, and retention of feeding skills could be assessed using a within-subjects design. One diet group was fed fish initially, and then had their diets switched to worms. A second diet group was fed worms initially, and had their diets switched to fish. Following the diet-switching phase, both groups were re-tested for feeding skills on their initial prey type. A third group was fed a mixed diet throughout the duration of the study to compare with the single diet

groups. In addition to examining the ontogeny of feeding skills in these three groups, when possible I tested for the effects of sex, site, and litter on feeding behavior.

Although the adult diets of the snakes from McCafferty farm and Miller's marsh differ, neonatal diets are not known. It is assumed that most young garter snakes begin life feeding on earthworms and later shift to other prey (Carpenter, 1952; Fitch, 1965; Greenwell, Hall, & Sexton, 1984). However, to test whether snakes from the two sites differed at birth in either initial foraging ability, or ability to profit by feeding experience, I tested neonates born to mothers from both sites.

Method

Subjects and maintenance

Subjects were 106 neonatal garter snakes (54 males, 52 females) from 16 litters (M litter size = 6.6, range = 1-15) born in the fall of 1998 to mothers collected at McCafferty farm (n = 9 litters) and Miller's marsh (n = 7 litters). These snakes were the same as those used in the growth study described in Chapter 2 (See Appendix I for specific litters tested).

Procedure

At birth, neonates were assigned to diets of either fathead minnows, *Pimephales promelas*, (F group, n = 35 snakes), leafworms, *Lumbricus rubellus*, (W group, n = 41 snakes), or a combination of the two (FW group, n = 30 snakes). These prey species were chosen because they are a part of wild garter snake diets and they are readily available through commercial sources. A split litter and sex design was used, with individuals from each litter and sex randomly assigned as equally as possible across each diet group. At 14

and 17 days of age, prior to the feeding tests, all snakes were tested for responses to prey extracts (see Chapter 3).

The snakes were given six feeding tests (FT1 – FT6). The testing schedule for Experiment I is summarized in Table 4.1. At 20 days of age, each snake was offered its first live prey (FT1). During each feeding test, the FW group was tested on both prey types, presented in random order, with three days separating the two trials. FT2 was completed 80-85 days after FT1, with the snakes receiving 11 or 12 weekly meals in between testing sessions. FT3 was completed after another 80-85 days, with the snakes still feeding on their initial diets during this interval. Following FT3, the diets of the F and W groups were reversed. FT4, representing the first time these snakes encountered a new prey item, was conducted on the F and W groups only, three days following FT3. FT5 was completed 45 days following FT4, with the snakes receiving 11 or 12 meals in between testing sessions. To meet increasing dietary needs, all snakes were fed twice weekly between FT4 and FT5. To assess the snakes' retention for the original dietary experience, prey items were then reversed again back to each snake's original diet at FT6, which was completed within 3 days after FT5.

Testing took place indoors at an air temperature of approximately 25° C. Live prey items weighing 10-15% of each snake's body weight, were placed in petri dishes in the center of each snake's home cage for each one-hour test. Water and a shallow layer of dirt were placed in each dish for fish and worms, respectively. Tests were either videotaped with a Hi-8 camcorder (Sony CRD-VX3) or recorded on check sheets through live observation. The dependent measures recorded at each test are described in Table 4.2. In addition to these measures, descriptive measures including prey capture locations

Table 4.1: Experiment I prey feeding skills testing schedule for the three diet groups.

		Diet group		
		Fish (F)	Worm (W)	Mixed (FW)
Feeding test (FT)	Age (days)	Test prey	Test prey	Test prey
FT1	20	Fish	Worm	Fish & Worm
FT2	98-103	Fish	Worm	Fish & Worm
FT3	178-183	Fish	Worm	Fish & Worm
FT4	181-186	Worm	Fish	-
FT5	226-231	Worm	Fish	-
FT6	229-232	Fish	Worm	-

Table 4.2: Dependent measures used for each feeding test.

Measure	Definition
Approach latency	Number of seconds from start of trial to time snake's head crossed rim of dish.
Capture time	Number of seconds from end of approach latency to when prey is seized in the snake's jaws.
Handling time	Number of seconds from prey seizure to when prey is maneuvered into place such that it can be swallowed.
Swallowing time	Number of seconds from time snake begins side to side jaw movements (jaw walking), which pushes prey into the throat, until first post-ingestive tongue flick.
Total consumption time	Number of seconds from onset of capture to first postingestive tongue flick.

Note: These measures were also used in Experiments II and III.

(head, mid-body, or tail), prey orientation during swallowing (head, mid-body, or tail-first), and the number of times prey were dropped were recorded. Prey items were removed if snakes had not eaten by the end of each one-hour test, and additional tests of the same prey species were run every second or third day until the snakes ate.

Statistical analyses

Effects of sex, litter, and site on total consumption time and each feeding phase were tested at FT1 using a MANOVA. Sex and site were specified as fixed factors, with Litter treated as a random factor nested in site. Wilk's Lambda (λ) was used to test for multivariate effects of each of the three factors. Separate univariate F-tests were used to examine the effects of sex, site, and litter on each feeding phase and total consumption times.

Changes in overall consumption times between FT1, FT2, and FT3 were tested using repeated measures ANOVA. The F and W groups were compared with diet treated as the grouping variable, and the interaction testing for relationships between diet and test (FT1-FT3). Changes in latencies to complete each feeding phase at FT1, FT2, and FT3 were tested using repeated measures ANOVA. The FW group was also tested with repeated measures ANOVA, comparing FT1 - FT3 for its consumption times and latencies to complete each feeding phase on fish and worm, separately. The effects of diet switching (FT4 & FT5) on total consumption times and feeding phases were evaluated using Wilcoxon signed-ranks tests for the F and W groups separately. The final feeding test, FT6, was compared with FT3 using Wilcoxon signed-ranks tests to evaluate whether prey consumption times, or the phases comprising it, increased following diet reversals for the F and W groups. All data were normalized using natural log (+1) transformations.

Results

Feeding records indicated that the snakes ate nearly equal proportions of each prey type between feeding tests (see Diet and Growth section, Chapter 2). No significant changes were found in prey capture location or swallowing direction, or the number of times prey were dropped between testing sessions. The results below first cover litter, sex, and site effects on each feeding phase and total consumption times, followed by analyses of overall changes in prey consumption times and phases between FT1 and FT3, and the effects of diet on these changes. Following are results on diet switching (FT4 and FT5) and tests for retention of feeding skills (FT3 and FT6).

Litter, sex, and site effects

At FT1, there were no overall effects for litter ($\lambda = 0.354$, $F = 356.4$, $df = 5, 10$, $p = 0.10$), site ($\lambda = 0.755$, $F = 0.65$, $df = 5, 10$, $p = 0.668$), or sex ($\lambda = 0.939$, $F = 0.13$, $df = 5, 10$, $p = 0.982$). Univariate F-tests revealed marginal litter effects for capture time and total consumption time at FT1 (Table 4.3). All interactions between litter, site, and sex were not significant. A second MANOVA was run to determine whether the neonates from Miller's marsh and McCafferty farm differed in their abilities to consume fish or worms at their first feeding. Site and prey type (diet) were treated as fixed factors and litter was treated as a random factor nested within site. The interaction between site and diet was included to test for geographic variation in fish or worm feeding skills. The results from this MANOVA revealed a non-significant interaction between site and diet ($\lambda = 0.94$, $F = 0.99$, $df = 5, 74$, $p = 0.430$). No significant results were found for litter, sex, or site at FT2 or FT3. Therefore, data for these three factors were pooled for all subsequent tests. Descriptive statistics comparing approach latencies and total

Table 4.3: Results from MANOVA testing for litter, site, and sex effects on each prey consumption phase and total consumption times by neonatal garter snakes at their first feedings (FT1). Significant p-values are boldfaced.

Source	DV	df	Hypothesis MS	Error MS	F	p
Litter within Site	Approach	14, 78	1.94	1.78	1.09	0.377
	Capture	14, 78	4.75	2.55	1.86	**0.044**
	Handle	14, 78	1.34	1.16	1.16	0.325
	Swallow	14, 78	0.35	0.22	1.57	0.107
	total	14, 78	2.20	1.21	1.82	**0.051**
Site	Approach	1, 14	6.64	1.94	3.42	0.086
	Capture	1, 14	0.05	4.75	0.01	0.923
	Handle	1, 14	0.00	1.34	0.00	0.922
	Swallow	1, 14	0.01	0.35	0.04	0.842
	total	1, 14	0.09	2.20	0.04	0.844
Sex	Approach	1, 14	1.08	1.94	0.55	0.469
	Capture	1, 14	1.05	4.75	0.22	0.645
	Handle	1, 14	0.13	1.34	0.10	0.762
	Swallow	1, 14	0.10	0.35	0.28	0.602
	total	1, 14	0.22	2.20	0.10	0.756

consumption times by the snakes from the two sites at FT1 through FT3 are shown in Table 4.4.

Initial feeding experience

Total consumption times. Predatory experience played a significant role in the development of the snakes' feeding skills. The snakes in all three diet-groups showed improvements in overall prey consumption abilities following feeding experience (Figure 4.1). Appendix IV summarizes the results for total consumption times and each feeding phase for the three diet groups at FT1 through FT3. The summary statistics in Appendix IV include only those snakes that ate at each test period. Changes in latencies to complete the feeding phases and total consumption times are expressed as percentage differences between feeding tests. The percentages were obtained by dividing the difference in seconds between the two tests under comparison (e.g., FT1 minus FT2) by the number of seconds taken to complete the first test (FT1). The snakes in the F and W groups significantly decreased their prey consumption times between the three tests (Table 4.5). The F group reduced their fish consumption times by 44.5% between FT1 and FT2, and by 67.9% between FT1 and FT3. The W group reduced worm consumption times by 42.5% between FT1 and FT2, and by 71.3% between FT1 and FT3. The degrees to which consumption times decreased were the same between the F and W groups, as shown by a non-significant interaction between test and diet (Table 4.5). Total consumption times decreased between FT1 and FT2, but the difference did not reach significance ($p = 0.09$). A significant decrease in total consumption time was found between FT1 and FT3 ($p = 0.003$), and not between FT2 and FT3 ($p = 0.967$). The latter comparison (between FT2 and FT3) indicates that prey were consumed as rapidly as possible by 183 days of age

Table 4.4: Descriptive statistics (M ± 1SE) for approach latencies and total consumption times at FT1-FT3 by snakes from Miller's marsh (MM) and McCafferty farm (MF) in each diet group. See Table 4.2 for definitions of behavioral terms.

			Diet							
			F		W		FW-F		FW-W	
Phase		Site	n	M (SE) sec.	n	M (SE) sec.	n	M (SE) sec.	n	M (SE) sec.
Approach	FT1	MM	10	524.6(215.5)	19	563.4(185.2)	12	542.6(209.6)	13	1561.1(309.1)
		MF	8	1300.0(432.1)	14	424.4(93.0)	10	687.4(150.2)	11	788.8(327.6)
	FT2	MM	13	824.5(367.8)	16	963.3(281.6)	14	194.8(40.1)	11	555.0(308.3)
		MF	8	276.3(124.2)	10	459.3(293.2)	8	251.9(109.5)	9	750.4(315.6)
	FT3	MM	10	952.1(249.9)	11	478.9(186.5)	11	494.9(147.8)	11	231.2(63.6)
		MF	5	179.2(65.2)	4	498.8(229.0)	5	283.0(192.8)	5	453.0(189.0)
Total	FT1	MM	10	1229.0(374.8)	16	895.9(211.1)	12	1138.9(301.9)	13	882.9(308.1)
		MF	8	1144.3(758.4)	14	715.6(234.3)	10	961.8(218.6)	11	350.5(128.4)
	FT2	MM	13	389.0(146.3)		221.7(47.6)	14	372.9(90.8)	11	171.7(60.3)
		MF	8	758.4(308.3)	10	478.7(226.6)	8	361.5(114.8)	9	340.1(169.2)
	FT3	MM	10	498.9(113.3)	11	172.8(40.5)	11	375.8(150.2)	11	212.1(82.0)
		MF	5	445.2(206.0)	4	378.5(175.9)	5	249.4(35.4)	5	470.2(342.0)

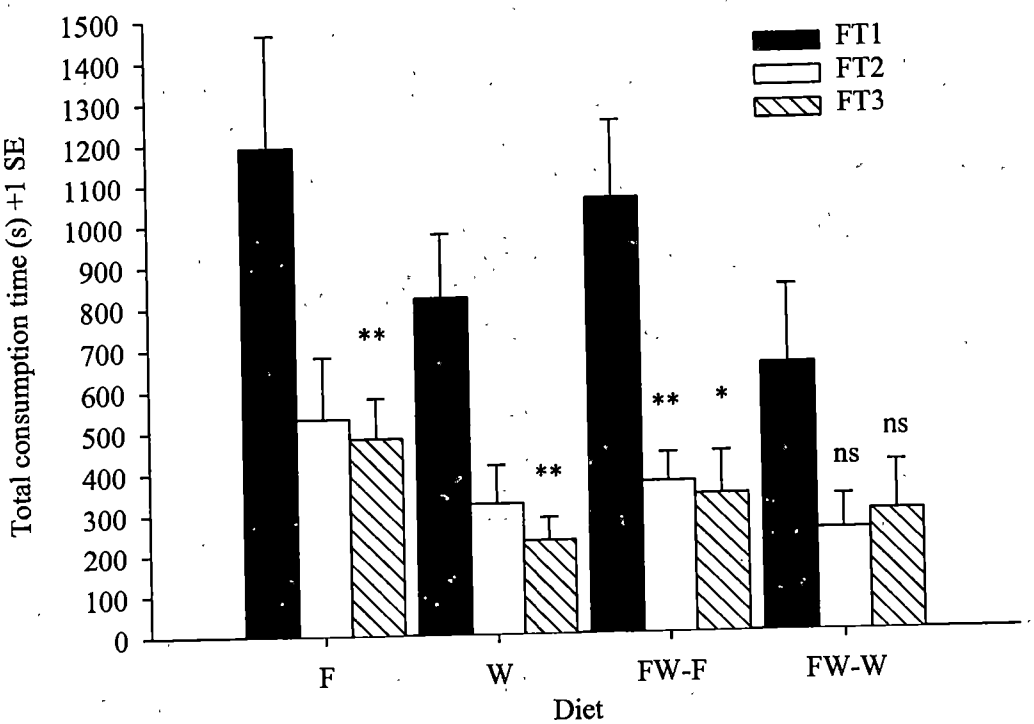


Figure 4.1: Mean (+ 1SE) total consumption times for garter snakes in each diet group at FT1, FT2, and FT3. F = Fish group, W = Worm group, FW-F = Mixed group (fish), FW-W = Mixed group (worm). * = $p < 0.05$, ** = $p < 0.01$ represent significant decreases in mean consumption times between FT1 compared with FT2 and FT3.

Table 4.5: Results from repeated measures ANOVAs testing for significant changes in total prey consumption times between the three test periods (FT1 - FT3) by snakes in the Fish (F), Worm (W), and Mixed (FW-F, FW-W) diet groups.

Diet group tested	Source	df	MS	F	p
	Test	2	7.25	6.42	**0.003**
*F & W groups	Test * Diet	2	3.71E-02	0.03	0.968
	Error	46	1.13		
**FW-F	Test	2	3.42	10.66	**0.001**
	Error	16	0.32		
**FW-W	Test	2	2.28	1.62	0.223
	Error	20	1.41		

* The F and W groups were grouped as diet, with the test * diet interaction testing for differences in the relative decreases in total prey consumption times between diet groups.
** Separate repeated measures ANOVAs were run for the Mixed (FW) diet group's total consumption times on fish (FW-F) and worms (FW-W). No interaction is tested because the snakes comprise a single (FW) diet group.

Snakes in the FW group also decreased their prey consumption times with feeding experience, but prey type affected the degree to which this occurred. The FW group consumed fish more rapidly after feeding experience, and the change was significant (see Table 4.5). Pairwise comparisons revealed significant reductions in fish consumption times between FT1 and FT2 (74.7%, $p = 0.01$), and between FT1 and FT3 (71.7%, $p = 0.033$). Fish consumption times between FT2 and FT3 were not significantly different ($p = 1.0$). Worm consumption times for the FW-W group did not decrease significantly overall (see Table 4.5), or between any of the three tests. However, worm consumption times by the FW-W group were relatively rapid at their initial feeding (see Figure 4.1).

To determine whether changes in total consumption times differed among the three diet groups, difference scores were calculated by subtracting the total seconds of FT2 from FT1. A one-way ANOVA on these scores did not reveal a significant diet effect ($F = 0.31$, $df = 3, 65$, $p = 0.816$). Difference scores comparing FT1 and FT3 revealed similar results ($F = 0.33$, $df = 3, 43$, $p = 0.802$).

Comparisons by feeding phase

Approach latencies. Results from repeated measures ANOVAs testing for the effects of feeding experience on changes in prey approach latencies are summarized in Table 4.6. Mean approach latencies to fish by the F group decreased between FT1 and FT2 (59.0%), and between FT1 and FT3 (63.3%). Approach latencies to worms barely decreased between FT1 and FT2 (0.5%), and decreased slightly between FT1 and FT3 (9.4%). The decreases in approach latencies across feeding tests were not significant for the F and W groups, and the test by diet interaction was not significant (Table 4.6).

Table 4.6: Results of repeated measures ANOVAs testing for significant changes in approach latencies, and capturing, handling, and swallowing times between the first three test periods by snakes in the Fish (F), Worm (W), and Mixed (FW-F, FW-W) diet groups.

Feeding phase	Diet group tested	Source	df	MS	F	p
Approach	F & W Groups	Test	2	1.47	0.99	0.379
		Test*Diet	2	0.38	0.26	0.775
		Error	46	1.48		
	FW-F	Test	1	4.46	2.54	0.110
		Error	16	1.76		
	FW-W	Test	2	7.05	2.42	0.114
		Error	20	2.91		
Capture	F & W groups	Test	2	18.59	7.42	**0.002**
		Test*Diet	2	0.59	0.24	0.791
		Error	46	2.51		
	FW-F	Test	2	18.97	16.39	**<0.001**
		Error	16	1.16		
	FW-W	Test	2	4.30	1.49	0.250
		Error	20	2.89		
Handle	F & W groups	Test	2	2.15	1.92	0.158
		Test*Diet	2	1.30	1.16	0.322
		Error	46	1.12		
	FW-F	Test	2	2.42	2.85	0.087
		Error	16	0.85		
	FW-W	Test	2	1.78	1.38	0.274
		Error	20	1.28		
Swallow	F & W groups	Test	2	5.91E-02	0.20	0.823
		Test*Diet	2	1.56	5.16	**0.009**
		Error	46	0.30		
	FW-F	Test	2	0.56	3.18	0.069
		Error	16	0.17		
	FW-W	Test	2	0.27	1.14	0.340
		Error	20	0.23		

Snakes in the FW group approached fish more rapidly between FT1 and FT2 (72.4%), but approach latencies to fish were not as reduced between FT1 and FT3 (41.6%). Approach latencies to worms by the FW group also decreased between FT1 and FT2 (31.7%), and FT1 and FT3 (78.2%), but the changes were not significant overall (see Table 4.6). Pairwise comparisons among the three tests did not reveal any significant differences. The three diet groups did not significantly differ from each another in approach times at FT2 ($F = 1.24$, $df = 3, 41$, $p = 0.307$), or FT3 ($F = 0.82$, $df = 3, 41$, $p = 0.491$)

Prey capture. Snakes feeding in single diets significantly reduced their prey capture times across the first three feeding tests (Table 4.6). Fish were captured more rapidly between FT1 and FT2 (53.0%), and between FT1 and FT3 (78.6%). Worm capture times decreased by 42.5% between FT1 and FT2, and by 79.6% between FT1 and FT3. Diet and testing period did not interact (Table 4.6). Multiple comparisons revealed significant decreases in capture times between FT1 and FT2 ($p = 0.044$) and FT1 and FT3 ($p < 0.001$). Capture times between FT2 and FT3 did not differ ($p = 1.0$).

Fish capture times decreased significantly for the FW group (Table 4.6). Fish were captured more rapidly at FT2 (90.1%, $p = 0.002$) and FT3 (94.3%, $p = 0.014$) compared with FT1. Worm capture times for the FW-W group decreased between FT1 and FT2 (63.6%) and FT1 and FT3 (53.9%), but the changes were not significant (Table 4.6). With the exception of the FW-W group, the capture phase was affected by feeding experience more than any other phase.

Prey handling. Prey handling times decreased slightly for the F group between FT1 and FT2, and increased between FT1 and FT3. Worms handling times by the W

group decreased between FT1 and FT2, and between FT1 and FT3, but the changes in handling times for both diet groups were not significant across test periods, and the test by diet interaction was not significant (see Table 4.6).

For the FW group, fish handling times increased between FT1 and FT2 , and between FT1 and FT3, but the changes were not significant overall (Table 4.6). Worm handling times decreased between FT1 and FT2, and between FT1 and FT3, but no significant changes were found (Table 4.6).

Prey swallowing. Swallowing times increased across testing periods for the F group and decreased for the W group. Fish swallowing times increased between FT1 and FT2 (22.0%), and between FT1 and FT3 (58.0%). Snakes in the W group swallowed worms more rapidly at FT2 than at FT1 (42.7%), and more rapidly at FT3 than at FT1 (30.5%). However, the changes in swallowing times were not significant (Table 4.6). The significant interaction between test and diet (Table 4.6) is due to F group's consistent increase in swallowing times, and the decreased swallowing times for the W group across testing periods.

For the FW group, fish swallowing times were nearly unchanged between FT1 and FT2, and increased between FT1 and FT3, but the differences were not significant (Table 4.6). Worm swallowing times decreased between FT1 and FT2 and between FT1 and FT3, and these changes to were not significant (Table 4.6).

The effects of diet reversal

Total consumption times. Due to snake mortality and food refusal, the sample sizes of the F and W groups diminished considerably between FT4 (181-186 days) and FT5 (226-231 days). The cause of mortality for each snake was not determined, but high

levels of garter snake mortality (>50%) in the first year has been reported (Jayne & Bennett, 1990). Nonparametric tests (Wilcoxon signed ranks test) were used to compare total consumption times and the feeding phases at FT3 and FT4, and at FT4 and FT5. The snakes' initial diets influenced their abilities to successfully switch to new prey. At their first feeding on worms (FT4), the snakes in the F group consumed worms as rapidly as the W group snakes did at FT2 (Figure 4.2), and consumed them as rapidly as they had fish at FT3 ($Z = 2.67$, $p = 0.79$). However, the snakes in the W group were much less successful at making an immediate adjustment to switching from worms to fish (see Figure 4.2). Comparing the W group's worm consumption time at FT3 with their first fish test at FT4 revealed a significant increase in the number of seconds taken to consume the prey ($Z = -2.34$, $p = 0.019$). However, they consumed fish more rapidly at FT4 (39.4%) than the snakes in the F group at their first feeding test with fish (FT1, Figure 4.2). This suggests that feeding on worms may have partially facilitated the snakes' ability to switch to a new diet. However, this comparison is confounded by maturational factors (e.g., physical development). The W group benefited from fish feeding experience, as shown by the 59.4% decrease in consumption time between FT4 and FT5, but this difference was not significant. This may be due to the fact that they were consuming fish as rapidly as possible given their body sizes and levels of feeding experience.

Comparisons by feeding phase

Descriptive statistics and statistical comparisons for each feeding phase are provided in Table 4.7. Snakes in the F group showed a slight decrease in worm approach time and the W group slightly increased their fish approach times by 7.3%, but the

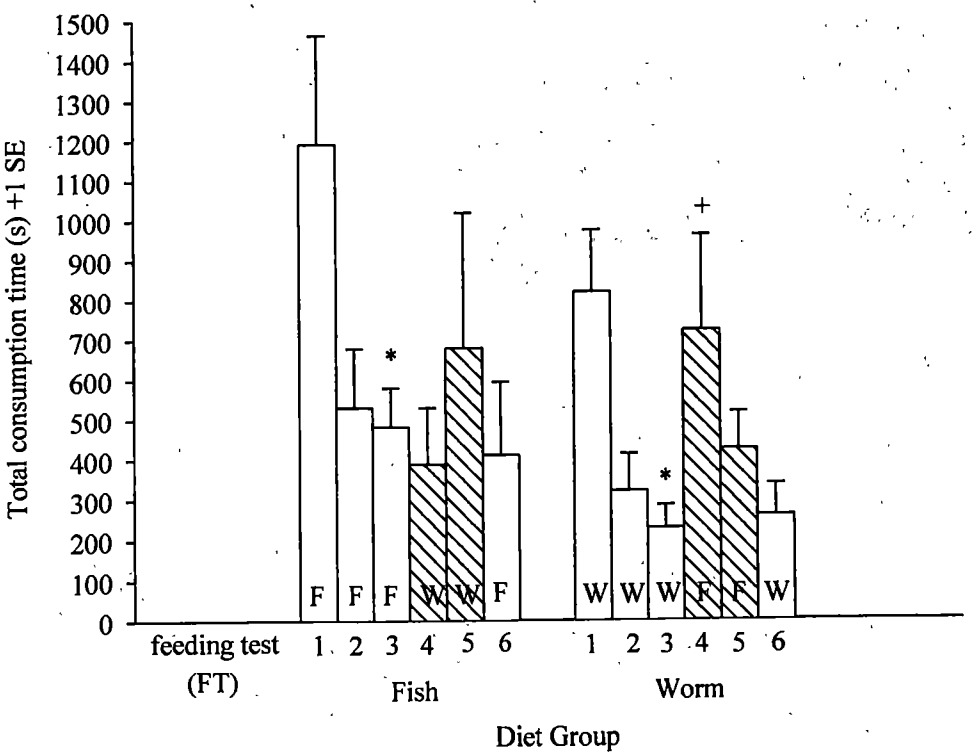


Figure 4.2: Mean (+ 1SE) changes in total consumption times as a function of diet (FT1-FT3) and diet reversal (FT4-FT5), and retention for feeding on initial diet (FT3 & FT6) in garter snakes. F = Fish prey, W = Worm prey. At FT4 diets were switched until FT5. Diets then returned to the initial one for FT6. * = $p < 0.01$ significant decrease in total consumption times between FT1 and FT3. + = $p < 0.05$ significant increase in consumption time between FT3 and FT4.

Table 4.7: Tests for the effects of diet reversal (FT4 - FT5) on each feeding phase and total consumption times for snakes in the F (Fish), and W (Worm) groups.

Feeding phase	Diet group	n	M (SE) sec. at FT4	M (SE) sec. at FT5	%diff	Z	p
Approach	F	5	682.4 (303.59)	657.6 (499.29)	-3.6	-0.14	0.893
	W	8	542.4 (158.95)	582.0 (204.53)	+7.3	-0.42	0.674
Capture	F	5	128.0 (55.87)	38.0 (21.97)	-70.3	-1.21	0.225
	W	8	633.3 (368.4)	240.6 (93.05)	-62.0	-0.84	0.401
Handle	F	5	21.4 (5.24)	17.4 (6.55)	-18.7	-0.55	0.581
	W	7	111.7 (27.14)	46.9 (11.2)	-58.0	-1.86	0.060
Swallow	F	5	67.0 (10.92)	104.0 (30.86)	+52.2	-0.94	0.345
	W	7	198.4 (28.36)	82.6 (12.1)	-58.4	-2.20	**0.028**
Total	F	5	216.4 (46.86)	169.6 (27.34)	-21.6	-1.21	0.225
	W	8	919.1 (376.26)	370.9 (87.0)	-59.4	-1.40	0.161

Note: Z = result from Wilcoxon signed ranks tests, p = p-value, FT4 = Feeding test 4, FT5 = Feeding test 5. FT4 was the first feeding test for the snakes on the reversed diet, and FT5 was the last test on the reversed diet, completed after11 or 12 feedings

changes were not significant. Decreases in worm capture times by the F group and fish capture times by the W group were not significant (Table 4.7). Worm handling times barely changed for the F group and the W group handled fish more rapidly at FT5 than at FT4, but neither change was statistically significant. Worm swallowing times actually increased for the F group, but the change was not significant, and fish swallowing times decreased significantly (58.4%) for the W group (Table 4.7).

Memory for initial diet

Total consumption times. Reversing the diets of the F and W groups did not appear to affect their abilities to detect and consume the prey on which they had initially fed. Total prey consumption times between FT3 and FT6 did not significantly change for either diet group (see Figure 4.2, Table 4.8). Fish consumption times slightly decreased between FT3 and FT6, and Worm consumption times increased slightly, but the changes were not significant.

Comparisons by feeding phase

The snakes showed no deterioration of ability to detect their first experienced prey. Approach latencies between FT3 and FT6 decreased for the F and W groups, but the changes were not significant (Table 4.8). Capture times also remained relatively unchanged between FT3 and FT6 for the F and W groups. Fish handling times increased for the F group, and worm handling time decreased, but neither change was significant. Swallowing times also did not significantly change between FT3 and FT6 for the F and W groups.

Table 4.8: Tests for retention of feeding skills in snakes from the F (Fish), and W (Worm) groups. Tests compare feeding phases and total consumption times at FT3 and FT6.

Feeding phase	Diet group	n	Mean(SE) sec. at FT3	Mean(SE) sec. at FT6	%diff	Z	p
Approach	F	8	505.3 (168.42)	287.4 (121.66)	-43.1	-1.26	0.208
	W	12	447.7 (164.86)	296.5 (108.38)	-33.8	-0.86	0.388
Capture	F	8	316.0 (112.23)	232.0 (185.44)	-26.6	-.98	0.327
	W	12	134.5 (60.32)	163.7 (80.8)	+21.7	-0.08	0.937
Handle	F	8	52.8 (19.26)	79.4 (41.56)	+50.4	-0.49	0.624
	W	12	31.6 (10.09)	17.1 (3.92)	-45.9	-1.49	0.136
Swallow	F	8	107.9 (24.63)	99.4 (22.97)	-7.9	-0.28	0.779
	W	12	67.8 (11.41)	80.6 (18.2)	+18.9	-0.63	0.530
Total	F	8	476.6 (120.81)	410.8 (183.45)	-13.8	-0.56	0.575
	W	12	233.8 (65.78)	261.3 (84.44)	+11.8	0.00	1.0

Note: Z = result from signed ranks test, p = p-value, FT3 = Feeding test 3, FT6 = Feeding test 6. FT3 was the last feeding test completed for snakes feeding on their initial diet, and FT6 was done following the diet reversal period.

Discussion

Experience played an important role in the development of feeding abilities in garter snakes through the first 8 months, and the changes that took place persisted. The importance of feeding experience was especially evident during the first 40 to 45 days, as evidenced by marked decreases in prey consumption times between FT1 and FT2. However, initial diet influenced the snakes' abilities to switch to new prey items. Feeding on fish, which can be relatively difficult for neonatal *T. sirtalis* to consume (Halloy & Burghardt, 1990), may facilitate switching to prey such as worms. Feeding on worms may have impeded the W group's ability to switch to fish, as prey consumption times increased significantly between FT3 and FT4 for this group. After 11 to 12 feedings on fish, the W group decreased its mean fish consumption time to a level comparable to the F group at feeding tests 1 and 2. Therefore, feeding experience played an important role in the ontogeny of feeding skills beyond the first 40 to 45 days. This may be the case even in wild, adult *T. sirtalis* that encounter novel prey species. It is unlikely that morphological differences accounted for the facilitation and interference effects found in the F and W groups, respectively. The snakes in the two diet groups did not significantly differ in SVL, mass, or head dimensions when diet switching took place (see Chapter 2, Growth and Morphology).

This experiment extends the findings of Burghardt and Krause (1999), where neonatal *T. sirtalis* were tested at birth and after 11 or 12 feedings. It appears that, with the exception of the W group's first feeding on fish, prey consumption times reach their lowest levels for young garter snakes after only 11 or 12 feedings. Also, similar to the

results reported by Burghardt and Krause (1999), prey consumption phases were differentially affected by feeding experience.

On average, prey approach latencies decreased across feeding tests, but the changes were not significant. This result was unexpected, given the significant decreases in prey approach latencies for the F and W groups tested in Burghardt and Krause (1999). This difference between the two studies may be due to the lower sample size used in the present study. Although there was a general trend toward decreasing prey approach latencies, the variability was much greater for approach latencies than in Burghardt and Krause (1999). Environmental conditions were nearly identical, and the same observer recorded data in both studies, thus ruling out the possibility of differences due to room temperature, caging, or the data recorder. One notable difference is that different species of fish were used in the two studies. The fish used by Burghardt and Krause (1999) were mosquito fish (*Gambusia affinis*), which has a dark dorsum and a white/gray underside. Due to low availability of *Gambusia* at the time of this study, we used fathead minnows (*Pimephales promelas*) instead, which were a special strain that had a light orange dorsum and a white/gray underside. Fish were size matched in proper proportions to snake body sizes, but the differences in coloration may have had different effects on approach latencies. However, this does not explain why approach latencies to worms did not decrease with feeding experience in this study. Motivational factors are unlikely, since snakes in both studies were tested at the same ages.

The snakes in the F, W, and FW-F group decreased their capture times significantly between FT1 and FT3. Burghardt and Krause (1999) report the same result in their study. The amount of time taken to capture prey exceeded that of handling and

swallowing prey. Decreased capture times accounted for the majority of the reductions in total consumption times between feeding tests for each diet group. Chemosensory detection of prey, as well as visual and tactile cues probably interact as prey capturing skills develop. *T. sirtalis* uses a fairly unspecialized tactic for capturing fish. When preying upon fish, aquatic specialist species such as *T. melanogaster* and *T. couchii* rely heavily on visually guided and direct strikes toward prey, whereas *T. sirtalis* uses an "open-mouth search" tactic (Drummond, 1983; Halloy & Burghardt, 1990). The open-mouth search is characterized by lateral movements of the head with the jaws open (Drummond, 1983; Savitsky & Burghardt, 2000), and prey seizure is probably facilitated by tactile and visual cues. In contrast to *T. melanogaster*, fish capturing tactics by neonatal *T. sirtalis* appear random, as orientation toward prey is much less direct and precise. However, fish capture times decreased significantly in this study, and Halloy and Burghardt (1990) report superior fish capturing abilities by adult *T. sirtalis* in comparison to yearling and neonatal conspecifics.

In my study, I did not record systematically record whether open-mouth searching occurred. However, I observed open-mouth searching quite frequently among the snakes in the F and FW-F groups at their first feeding trials. Open-mouth searching by the neonates was prolonged on occasion and often resulted in the snakes withdrawing from the water dish, especially if prey remained motionless and direct contact was not made. With feeding experience, it appeared that open-mouth searching became more directed and fewer lateral head movements were made. Unfortunately I can not provide much detail on this aspect of prey capturing behavior, but further work could closely examine the ontogeny of open-mouth searching behavior by *T. sirtalis*.

Worm swallowing times by the W and FW-W groups decreased in the present study, but not significantly. Burghardt (1978) recorded worm-swallowing latencies by 13 newborn *T. sirtalis* and found a consistent and rapid decline in swallowing times during each of their first 8 feedings. The W group in Burghardt and Krause (1999) significantly reduced the time it took to swallow worms. However, the swallowing times reported in Burghardt and Krause (1999) were greater at their initial feedings (131.2 s) than in my study (66.8 s). Comparisons of fish swallowing times for the F groups of both studies are complicated by the fact that different fish species were used in Burghardt and Krause (1999) and in this experiment. The fathead minnows generally took longer to swallow, probably due to different head sizes of the two species.

As hypothesized, the snakes showed no decrement in feeding skills for their initial prey following food switching. Comparisons between FT6 and FT3 for both diet groups yielded no significant changes in total consumption times or any of the feeding phases. Thus, it appears that experience plays a long lasting role in the early ontogeny of predatory behavior in *T. sirtalis*. This result also confirms that prey consumption times asymptote for snakes of this age. However, the amount of feeding experience on the initial diet versus the reversed diet was unbalanced. The snakes in the F and W groups had twice as much experience feeding on their initial prey species than they had with a new species. Including prey eaten during the feeding tests, the snakes in both groups had at least 25 meals (1 prey item/meal) between FT1 and FT3, and an average of only 13 meals (1 prey item/meal) on the reversed diet (FT4 - FT5). The results comparing FT3 and FT6 may have differed had the snakes eaten an equal number of meals on the

reversed diet. In Experiment II, I balanced and reduced the number of feedings on each prey species prior to and during the diet-switching phase.

Experiment II: Role of Early Learning and Retention in Feeding Behavior

Experiment I revealed the importance of experience on the development of garter snake feeding behavior through the first 8 months of life. Predatory skills, as measured by latencies to complete each feeding phase, reached a plateau by FT3 for snakes feeding at equal frequencies. Diet switching did not seem to result in any long-term decrement in feeding efficiency. However, snakes in the F and W groups fed on their initial prey for 5 months prior to having their diets reversed. If the amount of initial feeding experience on a single prey type were reduced it is possible that, in contrast to Experiment I, the retention for feeding skills on the initial prey would be reduced. On the other hand, it is possible that even if a prey type is only consumed infrequently, garter snakes will retain their feeding skills for the prey indefinitely. To test this, the second experiment used only F and W groups that experienced dietary switches after fewer feedings on their initial prey species.

Method

Subjects and maintenance

Subjects were 31 neonatal *T. sirtalis* (14 males, 17 females) from six litters (M litter size = 5.2, range = 2 - 9) born to females at Miller's marsh (n = 4 litters) and McCafferty farm (n = 2 litters) (see Appendix I). Housing conditions were the same as those described for neonates in Chapter 2.

Procedure

At birth, the snakes were divided into two diet groups, F (n = 13 snakes) and W (n = 18 snakes). Diet group assignment was balanced as evenly as possible across sexes and litters. At 15 days of age, the snakes were tested at their first meals (FT1) and were then fed weekly on their designated diet. Five feeding tests were completed (see Table 4.9). FT1 and FT2 were done to test for experiential effects on each prey type. FT3 and FT4 were done to test for the effects of diet switching and learning to handle novel prey, and FT5 tested the snakes' retention for consuming the initial prey species. Six feedings were completed between FT1 and FT2. Diets were switched at FT3 (completed 3 days after FT2) and six feedings followed until FT4. FT5 was completed 3 days after FT4. All tests were complete when the snakes reached 105 days. A single observer recorded all data live using the same dependent measures as in Experiment I.

Statistical analyses

The small sample size used in Experiment II, and the high degree of variability and unequal variances found in the data set, limited the types of analyses that could be conducted with the data. I used Wilcoxon signed-ranks tests to compare both diet groups separately. The variability in the capturing, handling, and swallowing times was high at each test. Also, similarly to Experiment I, handling and swallowing times were relatively unaffected by feeding experience. Because of this, analyses were conducted on approach latencies and total consumption times only. Wilcoxon signed-ranks tests were used to compare approach latencies and total consumption times at FT3 and FT4 (reversal tests), and at FT2 and FT5 (retention tests).

Table 4.9: Experiment II prey feeding skills testing schedule for both diet groups.

		Diet Group	
		Fish (F)	Worm (W)
Feeding test (FT)	Age (days)	Test prey	Test prey
FT1	15	Fish	Worm
FT2	57	Fish	Worm
FT3	60	Worm	Fish
FT4	102	Worm	Fish
FT5	105	Fish	Worm

Results

Initial feeding experience

Total consumption times. The snakes in the F group significantly decreased (62.3%) their total fish consumption times after only six meals (Table 4.10). However, the snakes in the W group did not show a significant decrease in total worm consumption times between FT1 and FT2 (Table 4.10, Figure 4.3A). Worm consumption times increased slightly (13.4%) between FT1 and FT2. However, their total consumption times at FT1 were already fairly low (399.1 s). This was considerably lower than the mean worm consumption time found for the W group in Experiment I (819.4 s). Thus, the lack of any significant decrease in worm consumption times may be due to a ceiling effect already evident in the snakes' feeding skills at FT1.

Approach latencies. Mean approach latencies did not decrease significantly for the F group between FT1 and FT2 (Table 4.10, Figure 4.3B). However, the snakes in the W group approached worms significantly faster at FT2 than at FT1 (Table 4.10, Figure 4.3B).

The effects of diet reversal

Total consumption times. Total consumption times between FT2 and FT3 did not significantly differ for the F group ($Z = -1.75$, $p = 0.08$). Thus, the snakes in the F group consumed their first worms at FT3 at the same rate as they had consumed fish following feeding experience. Total worm consumption times were not significantly different between FT3 and FT4 (Table 4.10, Figure 4.3A). Thus, the six worm feedings completed by the F group did not result in any significant change in total worm

Table 4.10: Results of tests for changes in approach latencies and total consumption times by snakes in the F (Fish), and W (Worm) groups after initial feeding experience, during diet reversal, and when prey were switched back to initial diets.

	Feeding phase	Diet group	n	M (SE) sec.	M (SE) sec.	%diff	Z	p
				FT1	FT2			
Initial FT1-FT2	Approach	F	8	900.0 (386.78)	286.3 (102.76)	68.2	-0.84	0.401
		W	16	436.8 (77.26)	120.3 (23.23)	72.5	-2.84	**0.004**
				FT1	FT2			
Initial FT1-FT2	Total	F	8	783.9 (233.61)	295.5 (71.59)	62.3	-2.24	**0.025**
		W	15	399.1 (80.09)	452.6 (126.78)	-13.4	-0.41	0.683
				FT3	FT4			
Reversal FT3-FT4	Approach	F	5	135.6 (56.33)	259.6 (122.18)	-91.4	-1.21	0.225
		W	5	97.6 (43.23)	64.2 (22.91)	34.2	-0.41	0.686
				FT3	FT4			
Reversal FT3-FT4	Total	F	5	830.4 (483.42)	381.6 (281.48)	54.0	-1.21	0.225
		W	5	1065.2 (539.62)	441.2 (132.06)	58.6	-0.67	0.50
				FT2	FT5			
Memory FT2-FT5	Approach	F	6	269.8 (125.81)	472.7 (370.29)	-75.2	-0.11	0.917
		W	7	122.9 (42.97)	237.7 (133.12)	-93.4	-0.85	0.398
				FT2	FT5			
Memory FT2-FT5	Total	F	6	253.8 (85.9)	288.8 (65.85)	-13.8	-1.99	0.460
		W	7	429.9 (230.54)	152.0 (31.83)	64.6	-1.18	0.237

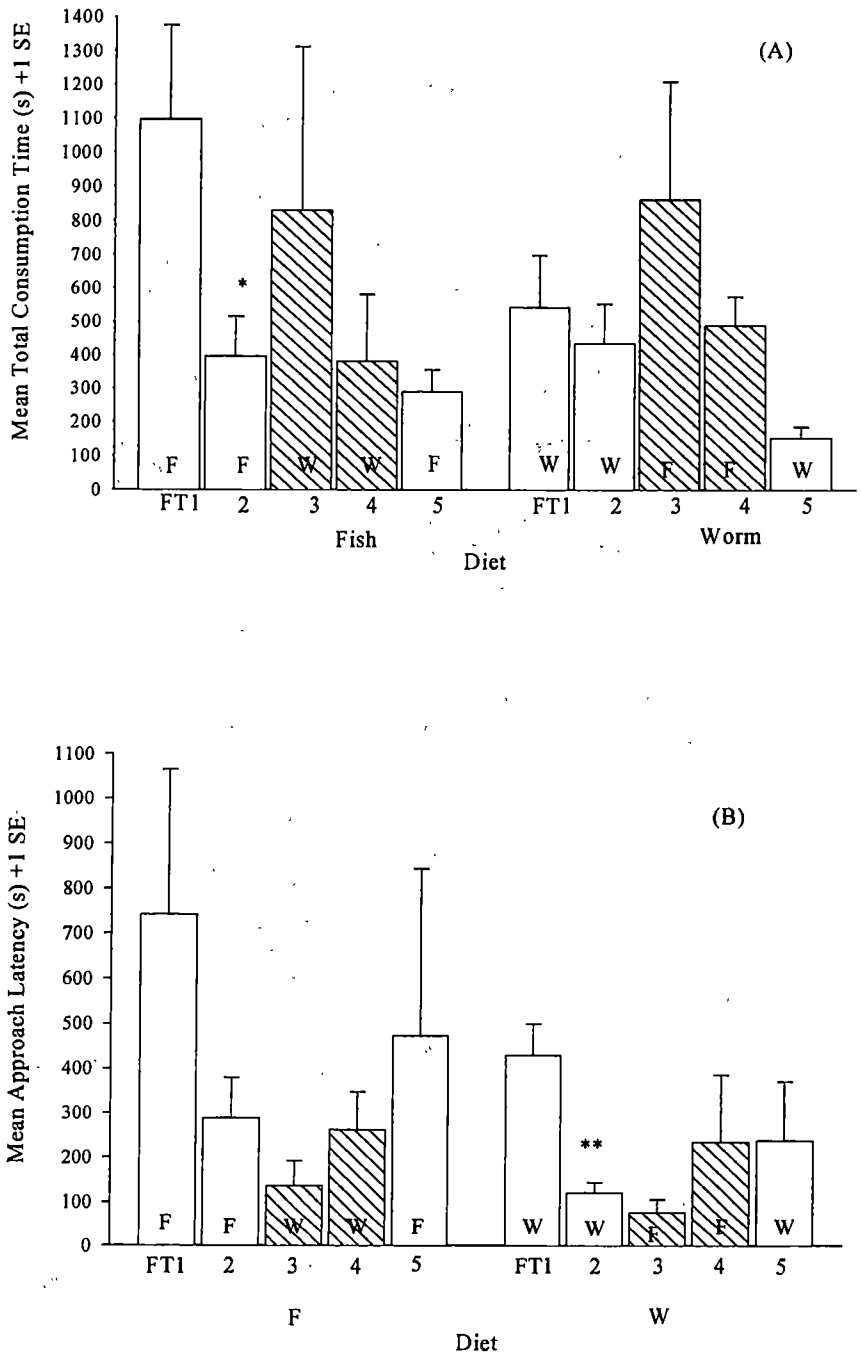


Figure 4.3: Mean (+SE) total consumption times (A) and approach latencies (B) for garter snakes in both diet groups at FT1 through FT5. Open bars = initial prey, shaded bars = switched prey. FT = feeding test. F = fish prey, W = worm prey. At FT3 diets were switched until FT4. Diets returned to the initial ones at FT5. * = < 0.05, ** = < 0.01 mean decreases between FT1 and FT2.

consumption time. Similarly, total consumption times between FT2 and FT3 did not significantly differ for the W group ($Z = -0.70$, $p = 0.484$). Thus, the interference effect found in Experiment I was not replicated here. However, comparisons between Experiments I and II are of limited use, as the sample size in Experiment II was very low. Total fish consumption times between FT3 and FT4 were not significantly different for the W group (Table 4.10, Figure 4.3A).

Approach latencies. Approach latencies between FT3 and FT4 did not change for either diet group (Table 4.10, Figure 4.3B).

Memory for initial diet

Total consumption times. No significant differences in total consumption times between FT2 and FT5 were found for either the F or W groups (Table 4.10, Figure 4.3A). Thus, the six-week period of feeding on the opposite diet did not result in a decrement in feeding skills for either group.

Approach latencies. Prey detection ability was unchanged after diet switching. Approach latencies to fish and worms did not differ between FT2 and FT5 for the F and W groups, respectively (Table 4.10, Figure 4.3A).

Sample size and statistical power

The small sample size used in this study resulted in low statistical power. A significant decrease in total fish consumption time was found between FT1 and FT2. However, the power of this test was calculated using the SamplePower option available on SPSS version 10.0. Power was estimated at 0.60 for the sample size of eight snakes. A sample size of 20 would have increased power to 0.97 given the means at FT1 and FT2 and the standard error of the difference between the two tests. The diet reversal phase

showed high mean decreases in total consumption times between FT3 and FT4 for both diet groups (Table 4.10). However, the differences were not significant. For the F group, power was estimated at 0.13, and a sample size of 60 would have increased power to 0.96. For the W group, power was estimated at 0.11, and a sample of 80 would have increased power to 0.96. The purpose of this study was to test for differences among samples, but these were rarely detected because of low sample size. Individual differences were substantial and are described in the following section.

Individual variation in feeding abilities

I found differences within diet groups in total consumption times and approach latencies. However, the non-significant results may have been due to the amount of variability in the data, although means differed considerably. For example, capture times decreased markedly between FT3 and FT4 for both diet groups, but neither difference was significant. Because rates of learning can vary so much among individuals, and repeatability may be low, it is important to consider individual and litter differences across all feeding tests. The small sample size of this study may not have provided the resolution needed to detect robust, group differences related to early feeding experience. One important consideration is whether individual differences in prey consumption abilities are consistent across feeding tests and prey types. To test this, averages across all five feeding tests were computed for each snake. Within each diet group, the snakes with the lowest and the highest averages were identified (Figure 4.4A-B).

The mean total consumption times for the five feeding tests differed considerably between the two snakes in the F group, 1729E (M total consumption time for FT1-FT5 = 1042.2 s) and 1723F (M total = 178.6 s). Compared to 1729E, feeding experience

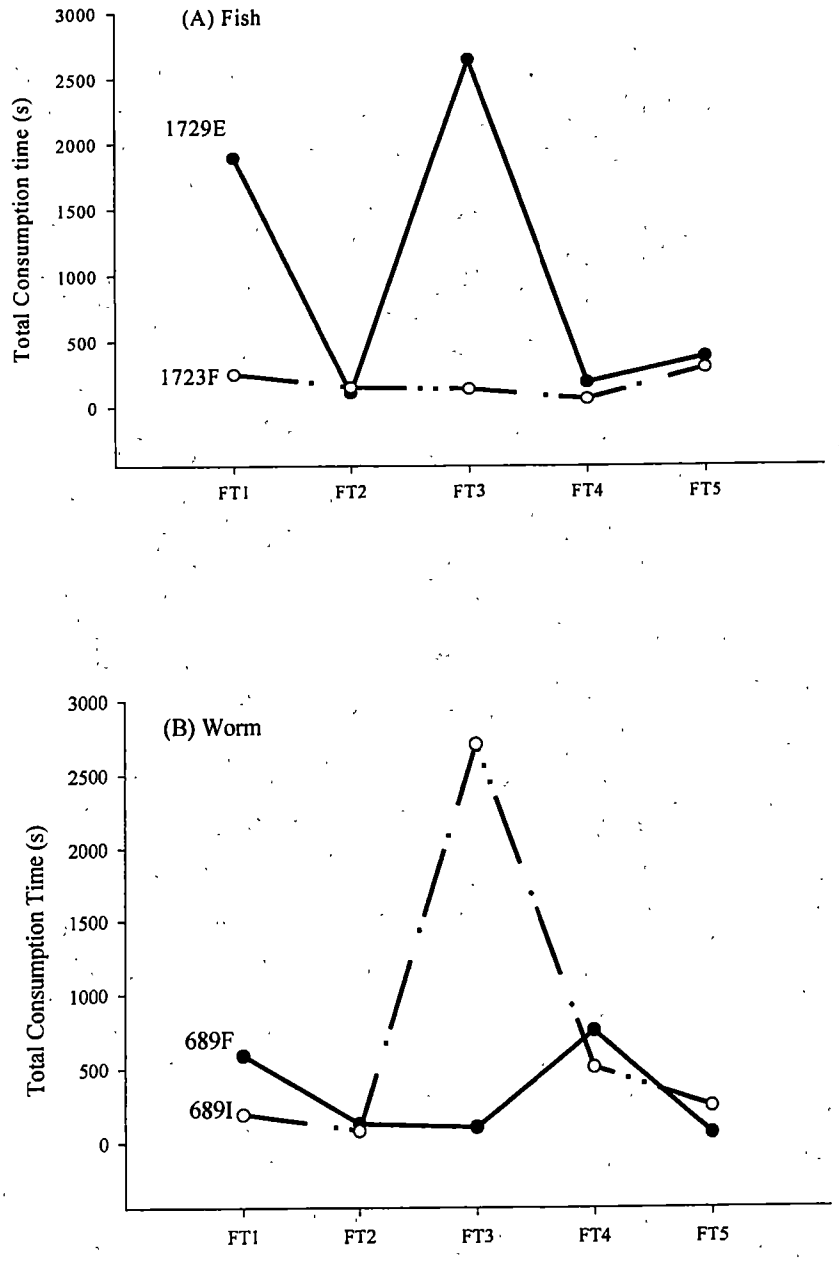


Figure 4.4: Individual differences in total consumption times between FT1 and FT5 for two snakes in the Fish group (A), and two snakes in the Worm group (B).

appeared to have little effect on 1723F's ability to consume prey, as his consumption time was brief at FT1 (248.0 s) and FT5 (296.0 s), with little variation among tests (Figure 4.4A). Experience appeared to be more crucial to the development of feeding in 1729E, or she had a bad day. Her consumption time at FT1 was relatively long (1892.0 s), and decreased considerably by FT2 (107.0 s). When 1729E's diet was switched to worms, her consumption time increased to levels comparable to FT1 (2644.0 s), but again decreased to levels comparable to 1729F at FT4 following feeding experience with worms. The differences between these two individuals may be related to the novelty of the prey items. Chemical preferences appear to be unrelated to these differences, because both snakes had tongue-flick attack scores of 0 to both fish and worm stimuli prior to FT1.

Snake 689I had a very high mean for the five tests (746.8 s) and 689F had a relatively low mean (329.2 s). Both snakes ate worms more rapidly at FT2 than at FT1 (Figure 4.4B). However, 689I took a relatively long time to consume the fish at FT3 (2703.0 s), but benefited considerably from fish feeding experience (FT4 = 511.0 s). 689F rapidly consumed a fish at her first feeding at FT3 (105.0 s, Figure 4.4B). The differences between these two snakes may be related to the novelty of the prey, which is similar to what was found for the two snakes in the F group. The differences between the two snakes in the W group at FT1 were not as great as between the two snakes in the F group. This may be due to the fact that worms are a staple of garter snake diets. However, the great disparity between 689F and 689I in consumption times at FT3 may be due to individual differences in response to a new type of prey.

Discussion

Feeding experience appeared to play a more crucial role in the development of predation on fish than on worms. The mean time to consume fish was considerably higher than the mean time to consume worms at FT1. In contrast, the snakes tested in Experiment I consumed both prey species at similar rates at FT1. At FT2, fish and worm consumption times were nearly equal. Approach latencies to both prey types decreased dramatically between FT1 and FT2, with the W group showing the greatest degree of change. Diet reversals did not have a statistically significant effect on eating a new prey item. However, mean total consumption times increased between FT2 and FT3 for both diet groups, and decreased following the six feedings after FT3 for both diet groups. Detection and consumption skills on initial prey items appeared to remain intact after diets were switched, as none of the measures increased at FT5 compared to FT2. This is consistent with the results from Experiment I, where diets were switched at a later age. Thus, it appears that very little experience is required before the response to a prey item is relatively "fixed" within the feeding repertoire of *T. sirtalis*.

Individual differences were considerable in this study, and appeared to be influenced by the type of prey consumed. Feeding experience may be more important for some snakes than others. Fish consumption times decreased significantly between FT1 and FT2. However, as Figure 4.4A shows, one subject consumed fish rapidly at FT1 whereas another took considerably longer, but these differences were reduced by FT2. The same pattern was repeated between FT3 and FT4. A similar finding was apparent for the two snakes in the W group (see Figure 4.4B), but only applied when the snakes switched to fish at FT3. Substantial individual differences in the development of prey

feeding skills were described in Experiment II. Chemosensory responses (Burghardt, 1975), and antipredator behavior (Brodie & Russell, 1999) are also known to vary greatly among individual snakes. Some snakes may require very little experience in order to efficiently detect, capture, handle, and swallow their prey. Snakes that require more feeding experience to acquire foraging skills may be at a relative disadvantage, especially if snakes that are efficient foragers from birth are also successful at switching to novel prey. This was the case for the individual snakes described in Experiment II. Although *T. sirtalis* is a prey generalist, some individuals may be very slow to eat upon their first opportunity, or to incorporate novel prey into their diets. These differences could have considerable importance in natural populations. They may form the basis for food resource partitioning, long term success in feeding, predator avoidance, and ultimately, survival and reproduction. Larger samples with a greater number of litters could be used to determine the genetic basis for these differences, and laboratory and field studies could determine the consequences that individual variation in learning abilities have in nature (see below).

Experiment III: Site Variation in Adult Predatory Skills

With some exceptions, feeding experience resulted in significant decreases in prey consumption times for the snakes tested in Experiments I and II, and these decreases were often related to the type of prey consumed. Following the completion of Experiment I of this chapter, I decided to devote the majority of the 1999 field season to examining the feeding skills of wild-caught adult snakes from both sites on Beaver Island. If feeding experience has long term effects on adult predatory behavior, then differences in consuming prey should reflect differences in natural diets. If differences exist in the

abilities of the adult snakes from the two sites to capture and consume prey, these could be due to either genetic differences between the sites or to the feeding histories of the individual snakes (Burghardt and Schwartz, 1999). Due to the close proximity of the two sites, I reasoned that any differences in prey feeding skills would be due to experience rather than to genotypic differences between sites.

Method

Subjects and maintenance

During May and June, 1999, 70 wild-caught adult garter snakes from both sites (Miller's marsh, n = 41 snakes; McCafferty farm, n = 29 snakes) were captured by hand and brought to the Biological Station. The snakes were sexed, weighed, measured (see Table 4.11) and scale clipped for later identification. All stomach contents were removed by gently palpating each snake's abdomen with the thumb. If ingested prey were detected, stomach contents were removed by gently pushing the prey forward through the stomach and gullet. Snakes were group housed by site in glass aquaria (46 x 91 cm), and water was available ad libitum. Temperature and lighting conditions were the same as those described for wild-caught adults in Chapter 2. Each snake was assigned to one feeding condition: Fish, Worm, or Frog. This was done randomly, with the constraint of balancing as best as possible across sex and site. Banded killifish (*Fundulus diaphanus*) were captured by seine along the north shore of Beaver Island, green frogs (*Rana clamitans)* were captured with dip nets from various ponds near the Biological Station, and earthworms (*Lumbricus terrestris*) were obtained from a local bait supplier.

Table 4.11: Mean (± 1SE) SVL, body weight, and total number of feeding tests completed for each prey item by males and females from Miller's marsh (MM) and McCafferty farm (MF).

Site		SVL (mm)	Weight (g)	Prey		
				Fish	Frog	Worm
MM	Males (n=22)	441.6(10.2)	33.9(2.1)	9	2	11
	Females(n=22)	497.4(17.2)	58.7(6.3)	6	8	8
MF	Males(n=9)	481.7(12.8)	49.2(2.9)	4	0	5
	Females(n=20)	512.9(14.8)	68.8(5.0)	3	9	8

Procedure

Snakes were allowed to acclimate to captivity for three to five days prior to testing. This, along with stomach content removal, served to standardize hunger levels as much as possible. One hour prior to testing, each snake was placed in a clear plastic cage (29 x 41 x 16 cm) with a paper towel substrate. Data were collected live, using the same behavioral measures as for neonates in Experiments I and II (see Table 4.2). Prey were weighed to the nearest 0.1 gram and placed into clear plastic bowls (150 x 65 mm) with an opaque paper strip surrounding the outside of the bowl to minimize visual cues.

The banded killifish was the largest species of fish that could be consistently captured by seine along the island shore. The fish offered were on average 8.0% (SE = ±0.01%) of snake body masses. Worm body weights were on average 9.0% (SE = ±0.01%) of snake body masses. The species of frog used for testing, and the body sizes of individual frogs, were chosen based on availability. The snake-prey body-size proportions of the frogs were not the same as those for fish and worm prey. Frogs of comparable sizes to fish and worms were not consistently found at Miller's marsh or in ponds near the biological station. Adult green frogs were abundant enough during the study period to use for testing. Frog body weights were on average 27.0% (SE = ±0.02%) of snake body masses.

Bowls holding fish or frog prey were filled to half with water, with a shallow layer of gravel at the bottom. Worms were placed in a shallow layer of dirt. To prevent the prey from escaping, lids were placed on top of the bowls with an opening (80mm diameter) cut in the center so that the snakes could enter (and chemical cues could

escape). If prey were not captured within one hour, trials were terminated and repeated the following day. Snakes that did not eat after three trials were released.

Statistical analyses

A MANCOVA was used to test for the effects of prey type (prey) on each feeding phase, and on total consumption times. Sex, site, and prey were treated as fixed factors. To control for the effects of snake and prey body sizes on all measures, SVL and prey weight were treated as covariates. No significant effect for sex was found so this factor was dropped from the model. Pairwise comparisons were used to compare differences in each phase and total consumption times among prey types. Descriptive statistics of non-transformed values revealed several outliers beyond 3 standard deviations, and the assumption of homogeneity of variances was not met for several measures. Transforming the data using natural log (+1) transformations resulted in normalized data with equal variances.

Results

Overall prey, sex, and site effects

Descriptive data were gathered for each feeding phase as well as total consumption times for each prey type for snakes from both sites (Table 4.12). SVL was a significant covariate ($\lambda = 0.67$, $F = 5.16$, $df = 5, 52$, $p = 0.001$), as was prey mass ($\lambda = 0.64$, $F = 5.98$, $df = 5, 52$, $p < 0.001$). Snout-vent length significantly covaried with capturing, swallowing and total consumption time. Prey weight significantly covaried with all phases except for approach time. A marginally significant effect was found for site ($\lambda = 0.83$, $F = 2.11$, $df = 5, 52$, $p = 0.078$) and a significant effect was found for prey ($\lambda = 0.31$, $F = 8.26$, $df = 10, 104$, $p < 0.001$). The univariate tests on each phase and total

Table 4.12: Mean (± 1 SE) differences (in sec.) among each prey type for all feeding phases and total consumption times for wild-caught adult garter snakes from Miller's marsh (MM) and McCafferty farm (MF).

			Approach	Capture	Handle	Swallow	Total
		n	M (SE) sec.	M (SE) sec.	M (SE) sec.	M(SE) sec.	M(SE) sec.
Fish	MM	15	594.5(103.4)	399.4(108.0)	53.3(12.2)	88.1(16.2)	540.8(109.5)
	MF	7	231.4(75.9)*	315.9(65.3)	64.6(38.1)	130.0(60.2)	510.4(146.2)
	Mean		413(89.7)	357.7(86.7)	59.0(25.2)	109.1(38.2)	525.6(127.9)
Worm	MM	19	711.6(145.3)	112.6(55.2)	13.7(3.3)	49.7(5.7)	170.3(53.5)
	MF	13	264.7(60.5)*	77.5(41.3)	10.3(2.2)	90.2(15.8)*	178.0(41.9)
	Mean		488.2(102.9)	95.1(48.3)	12.0(2.75)	70.0(10.8)	174.2(47.7)
Frog	MM	10	437.8(162.6)	376.0(146.6)	669.1(268.5)	1045.7(315.0)	1950.2(502.3)
	MF	9	344.8(96.1)	1000.0(889.3)	603.8(143.7)	1209.3(169.8)	2496.2(1019.4)
	Mean		391.3(129.4)	688.0(518.0)	636.5(206.1)	1127.5(242.4)	2223.2(760.9)

Note: * = $p < .05$ difference between sites.

consumption times for each factor gave similar results (Table 4.13). The significant site effect is explained by differences in swallowing times. Snakes from McCafferty farm took longer to swallow all three prey types. However, snakes from McCafferty farm approached prey more quickly than the snakes from Miller's marsh (Table 4.12).

Pairwise comparisons were made to determine which feeding phases differed among prey types (Table 4.14). Mean latencies to capture, handle and completely consume fish were significantly longer than for worms, but approach latencies and swallowing times did not differ. Frogs took significantly longer to handle, swallow, and completely consume than both fish and worms. Worm capture times were significantly faster than frog capture times. These results were not due to differences in relative head sizes, as head length was not a significant covariate.

Site effects

A second MANOVA compared all measures for each prey type separately, with site as a grouping variable. Sex was not a significant factor for any of these tests and was dropped from the model. SVL and prey weight were treated as covariates.

Fish. Overall, SVL significantly covaried with the amount of time taken to consume fish ($\lambda = 0.28$, $F = 7.29$, $df = 5, 14$, $p = 0.001$). All feeding phases were significantly affected by SVL except for approach latency (Table 4.15). Prey weight was also a significant covariate ($\lambda = 0.32$, $F = 5.84$, $df = 5, 14$, $p = 0.004$), with swallowing and total consumption times significantly affected by prey weight (Table 4.15). A significant effect for site was found for fish prey ($\lambda = 0.30$, $F = 6.52$, $df = 5, 14$, $p = 0.002$). However, although snakes from both sites captured, handled, and swallowed fish with equal proficiency (see Table 4.15), the snakes from McCafferty farm approached

Table 4.13: Results of MANCOVA testing for sex, site, and prey effects on each feeding phase and total consumption times in wild-caught garter snakes from Miller's marsh (n = 44) and McCafferty farm (n = 29).

Source	DV	df	MS	F	p
Covariate (SVL)	Approach	1	2.48	2.29	0.135
	Capture	1	5.06E-02	0.03	0.867
	Handle	1	1.73	1.61	0.209
	Swallow	1	0.62	1.60	0.211
	Total	1	9.78E-02	0.17	0.686
Sex	Approach	1	4.18E-02	0.04	0.845
	Capture	1	0.58	0.32	0.571
	Handle	1	2.16	2.01	0.161
	Swallow	1	0.50	1.31	0.257
	Total	1	1.05	1.78	0.188
Site	Approach	1	7.0	6.48	**0.014**
	Capture	1	8.33E-02	0.05	0.83
	Handle	1	0.44	0.41	0.524
	Swallow	1	2.83	7.35	**0.009**
	Total	1	0.57	0.96	0.332
Prey	Approach	2	0.11	0.10	0.906
	Capture	2	25.63	14.25	**<0.001**
	Handle	2	39.44	36.70	**0.001**
	Swallow	2	26.16	68.0	**0.001**
	Total	2	26.94	45.4	**0.001**
Error	Approach	58	1.08		
	Capture	58	1.80		
	Handle	58	1.08		
	Swallow	58	0.39		
	Total	58	0.59		

Table 4.14: Pairwise comparisons between each prey species for all feeding phases and total consumption times by wild-caught garter snakes from Miller's marsh and McCafferty farm. All significant *p*-values are boldfaced.

		Worm		Frog	
	Feeding phase	M diff(sec) fish-worm	p value	M diff(sec) fish-frog	p value
Fish	Approach	-75.2	1.0	21.7	1.0
	Capture	262.6	**<0.001**	-330.3	1.0
	Handle	47.0	**<0.001**	-577.5	**<0.001**
	Swallow	39.1	0.174	-1018.4	**<0.001**
	Total	351.4	**<0.001**	-1697.6	**<0.001**
				worm-frog	
Worm	Approach	-	-	96.9	0.414
	Capture	-	-	-592.9	**0.011**
	Handle	-	-	-624.5	**<0.001**
	Swallow	-	-	1057.5	**<0.001**
	Total	-	-	-2049.0	**<0.001**

Table 4.15: Results from MANOVAs testing for site effects on each feeding phase and total consumption times for each prey type.

Prey	Source	DV	Df	MS	F	P
Fish	Covariate (SVL)	Approach	1	0.24	0.28	0.601
		Capture	1	4.50	5.27	**0.034**
		Handle	1	5.38	4.65	**0.045**
		Swallow	1	5.38	28.28	**<0.001**
		Total	1	4.53	10.99	**0.004**
	Covariate (Prey weight)	Approach	1	0.43	0.51	0.484
		Capture	1	0.72	0.84	0.372
		Handle	1	1.60	1.38	0.255
		Swallow	1	6.50	34.16	**<0.001**
		Total	1	1.95	4.73	**0.043**
	Site	Approach	1	5.36	6.42	**0.021**
		Capture	1	0.66	0.78	0.389
		Handle	1	0.27	0.23	0.636
		Swallow	1	0.64	3.35	0.084
		Total	1	0.25	0.61	0.446
	Error	Approach	18	0.84		
		Capture	18	0.85		
		Handle	18	1.16		
		Swallow	18	0.19		
		Total	18	0.41		
Worm	Covariate (SVL)	Approach	1	9.32	10.01	**0.004**
		Capture	1	1.86	1.05	0.314
		Handle	1	3.44E-03	0.01	0.942
		Swallow	1	1.11	3.78	0.062
		Total	1	1.61	2.82	0.105
	Covariate (Prey weight)	Approach	1	3.30	3.55	0.070
		Capture	1	0.57	0.32	0.574
		Handle	1	2.70E-04	0.01	0.984
		Swallow	1	5.94E-02	.020	0.656
		Total	1	0.28	0.50	0.488

Table 14.5
(continued)

Worm Cont.	Site	Approach	1	7.33	7.87	**0.009**
		Capture	1	0.16	0.09	0.765
		Handle	1	0.44	0.68	0.417
		Swallow	1	1.50	5.12	**0.032**
		Total	1	0.15	0.27	0.609
	Error	Approach	27	0.93		
		Capture	27	1.77		
		Handle	27	0.64		
		Swallow	27	0.29		
		Total	27	0.57		
Frog	Covariate (SVL)	Approach	1	0.14	0.08	0.780
		Capture	1	0.74	0.21	0.664
		Handle	1	5.89	4.55	0.070
		Swallow	1	2.25E-02	0.16	0.704
		Total	1	9.29E-02	0.41	0.540
	Covariate (Prey weight)	Approach	1	0.20	0.12	0.739
		Capture	1	4.27	1.18	0.313
		Handle	1	1.17	0.90	0.373
		Swallow	1	0.39	2.72	0.143
		Total	1	0.39	1.73	0.230
	Site	Approach	1	4.67E-02	0.03	0.872
		Capture	1	3.71E-03	0.01	0.975
		Handle	1	0.82	0.63	0.452
		Swallow	1	0.22	1.54	0.254
		Total	1	7.41E-05	0.01	0.986
	Error	Approach	7	1.66		
		Capture	7	3.60		
		Handle	7	1.29		
		Swallow	7	0.14		
		Total	7	0.22		

fish more rapidly than did the snakes from Miller's marsh. Total fish consumption times did not differ between the two sites.

Worm. Snout-vent length covaried with the amount of time taken to consume worms ($\lambda = 0.49$, $F = 4.73$, $df = 5, 23$, $p = 0.004$), but prey weight was not a significant covariate ($\lambda = 0.79$, $F = 1.19$, $df = 5, 23$, $p = 0.345$). A significant site effect was found for worm prey ($\lambda = 0.36$, $F = 2.85$, $df = 5, 25$, $p = 0.036$). The snakes from McCafferty farm approached worms more rapidly than did the snakes from Miller's marsh, but the snakes from Miller's marsh swallowed worms more rapidly (Table 4.12). Total worm consumption times did not differ between the two sites (Table 4.15).

Frog. Snout-vent length did not covary with times taken to consume frogs ($\lambda = 0.34$, $F = 1.18$, $df = 5, 3$, $p = 0.474$). Prey weight was not a significant covariate either ($\lambda = 0.40$, $F = 0.90$, $df = 5, 3$, $p = 0.574$). Overall, site was not a significant factor for frog prey ($\lambda = 0.34$, $F = 0.52$, $df = 5, 5$, $p = 0.755$), and there were no differences for any of the feeding phases or total frog consumption times between the two sites (Table 4.15).

Discussion

The limited worm diet of the adult snakes from McCafferty farm apparently has no effect on their abilities to handle large bodied and difficult to handle prey such as frogs, and novel prey such as fish. The quicker swallowing times, especially for worms, by snakes from Miller's marsh may be due to their normally feeding upon larger prey. Morphological differences are unlikely to account for the quicker swallowing times by the snakes from Miller's marsh, as head size variation between sites was minimal (see Chapter 2). Also, the species of earthworm used in this experiment is not native to Beaver

Island and individuals are much larger than the native worms. Thus, the snakes from Miller's marsh may have had a slight advantage in worm swallowing performance over the snakes from McCafferty farm. Indeed, experience with more difficult prey (fish and frogs) may facilitate swallowing of less difficult prey and swallowing time was the only overall site difference found, with the snakes from Miller's Marsh swallowing all of their prey faster than the snakes from McCafferty Farm.

The rationale and results of Experiment III rest on the assumption that the natural diets of the snakes from the two sites differ. The results must be treated as tentative, as appropriate methods (e.g., radio telemetry) have not been implemented to determine the full wandering range of garter snakes at McCafferty farm. It is possible that the snakes from McCafferty farm have migrated to and from sites where amphibians are available. However, given their smaller mean body sizes (see Chapter 2) and because gut contents have consisted only of worms from several field seasons since 1991 (Gillingham and Burghardt, unpubl.), it is a fairly safe assumption that these snakes consume primarily worms. Furthermore, Graves, Halpern, and Gillingham (1993) recorded home range use by *T. sirtalis* at Jordan River, a site near McCafferty farm were the snakes specialize on earthworms, and found that healthy adult snakes remained near their capture sites and moved an average of only 40.4 m/day. If the home range usage by the snakes from McCafferty farm is comparable to the Jordan River snakes, they still would not encounter habitat similar to Miller's marsh. Genetic studies are needed to establish the extent, if any, of genetic differentiation between the sites.

In light of the results from Experiment I, the long-term importance of feeding experience on prey capture and handling may not be dependent upon the type of prey

consumed. Adult snakes from McCafferty farm were equally proficient at consuming frogs, presumably a novel prey type, as snakes from Miller's marsh. The same applied to feeding on worms, which are common to both sites, and fish, which are not present at either site. Based on Experiment I, and on Burghardt and Krause (1999), the primary hypothesis that I tested in Experiment III was that, based on their presumed feeding histories, snakes from Miller's marsh would handle frogs and fish more proficiently than snakes from McCafferty farm. Approach latencies to frogs were equal for snakes from both sites, suggesting that motivational factors were not a factor for this prey species. However, the adults from McCafferty farm approached worms and fish more rapidly than did the snakes from Miller's marsh, but capture times were not significantly different. Differences in reactivity could account for the slower approach times by the snakes from Miller's marsh. Also, the snakes from McCafferty farm may have adjusted more rapidly to captivity than the snakes from Miller's marsh, which is possible because the snakes from McCafferty farm could have been repeatedly handled and measured during class projects.

Feeding experience plays an important role in the ontogeny of feeding by young *T. sirtalis* (Burghardt & Krause, 1999; Halloy & Burghardt, 1990; Experiments I and II of my study). However, when adult body size is reached prey size may be a more important determinant of the amount of time taken to consume prey. Perhaps prey specific experience only manifests itself in a narrow range of relative prey sizes. In addition, the anti-predator behavior of prey may constrain the tactics that predators can use in capturing, handling, and swallowing their prey. The snakes from McCafferty farm consumed frogs with equal proficiency to the snakes from Miller's marsh, which suggests

that this may be the case. All frogs were swallowed rear end first, and no attempts at head-first ingestion were observed. The large body sizes of the frogs, and presumably their relatively high levels of strength, appear to require that the snakes subdue the posterior (leg) region of the frogs to prevent escape. Snakes from both sites did this on each trial in which frogs were eaten. If tested on dead (or smaller) frogs, the snakes from Miller's marsh may have handled and swallowed their prey more rapidly than the snakes from McCafferty farm. Captive *T. sirtalis* will consume dead prey (Arnold, 1978) and may scavenge in the field when provided the opportunity. However, consumption of live prey by wild *T. sirtalis* is probably much more frequent, and the ecological validity of Experiment III was increased with the use of live prey. Testing the snakes in laboratory conditions may have compromised the ecological validity of Experiment III. Foraging in aquatic habitats such as Miller's marsh may require skills that could not be expressed in my laboratory tests, such as detecting and subduing prey underwater.

General Discussion

Snakes and other reptiles have an often overlooked capacity to learn (see Burghardt, 1977). In Experiments I and II, neonatal garter snakes improved their foraging skills with feeding experience. There are many kinds of learning though, and these can be understood in terms of the ecological and evolutionary histories of the organisms studied (Shettleworth, 1993, 1998). The studies reviewed by Burghardt (1977) primarily covered operant, associative, and maze learning in many species of reptiles. Spatial learning and memory have been systematically studied in several reptilian species (see Day, Crews, & Wilczynski, 1999; Holtzman et al., 1999, refs. therein). Ford and Burghardt (1993)

review ecologically relevant research examining several types of learning in reptiles, including chemosensory identification of prey and predator avoidance.

Learning to detect, capture and consume prey encompasses a variety of sensory and behavioral changes. For example, the improvements in prey capturing abilities by the F, W, and FW groups in Experiments I presumably required the integration of chemical, visual, and tactile senses (although the predominant modality was not determined). The natural history *T. sirtalis*, with their broad geographical distribution and diverse diets, suggests that the benefits of being a dietary generalist far outweigh the costs. Their propensity to attack a wide variety of prey (Burghardt, 1969), and their abilities to rapidly acquire foraging skills on new prey, suggest that plasticity of foraging behavior in *T. sirtalis* is a primary factor in their success as a species.

Because live prey were used, the behavior of the fish and worms probably affected the results of the three experiments. Capture times may have decreased across test periods in Experiment I because the snakes were better able to detect prey movements with feeding experience. Burghardt and Denny (1983) found that prey movement, in addition to chemical cues, is an important factor that elicits predatory responses in *T. sirtalis*. Alternatively, the snakes may have been slow to approach their prey due to neophobia. However, this potential explanation probably would not apply to every snake, as neophobic responses to species-typical prey would be highly maladaptive. Furthermore, ingestively naive snakes attack prey odors, which indicates a natural propensity to move toward, rather than away from, prey.

Handling and swallowing times are almost certainly affected by whether prey are alive or dead. Decreases in handling and swallowing times may be facilitated by the

snakes' familiarity with how prey attempt to escape. Future work could compare the acquisition of feeding skills by snakes feeding on live or dead prey. Also, identifying changes and integration among sensory modalities (e.g., chemical, visual, tactile) as feeding skills increase would provide further, and more detailed, explanation for the development of successful foraging. Diets and test prey were randomly assigned without consideration of individual prey preferences in my experiments. Feeding experience and exposure to prey chemicals are known to alter prey preferences in *T. sirtalis* (Burghardt, 1992). Congenital and experience-based prey preferences were not tested in my study, but are important sources of plasticity of snake foraging behavior.

The retention of feeding skills

Following diet switching, the snakes did not show any decrement in feeding skills for prey comprising their initial diet. The concept of a 'memory window' has been developed by several investigators studying foraging behavior (e.g., Cuthill, et al., 1990; Hughes, et al., 1992; Valone, 1992). The 'memory window' refers to the "...duration of learned information or skills, for example in relation to food caches, harvest rate, prey handling time, or recognition of potential predators" (Mackney & Hughes, 1995, pp. 1241). Memory for prey toxicity could be added to this list, and has in fact been demonstrated in garter snakes (Burghardt, Wilcoxon, & Czaplicki, 1973; Terrick, Mumme, & Burghardt, 1995).

The memory window for prey feeding skills by young *T. sirtalis* appears to be at least 10 weeks, as feedings at FT6 were done with the same proficiency as feedings on the same prey at FT3 (Experiment I). The memory window for prey feeding skills is most likely much larger and warrants further study. Also, comparisons between generalist and

specialist snake species could be made. Memory windows for feeding on novel, atypical prey may be greater in *T. sirtalis* than in more specialized species such as *T. melanogaster*, or *T. butleri*.

Memory can be viewed from a functional, adaptive perspective. For example, Anderson and Schooler (1991) suggest that the probability of retrieving encoded information should be equal to the probability of the information being needed again. Experiments testing this hypothesis have largely been conducted with human subjects (e.g., Anderson, 1991), but the relationship between event recurrence and retrieval probabilities can be applied to nonhuman species (see Shettleworth, 1998). So far, the majority of the literature dealing with this relationship seems to be on memory for seed cache storage in birds (see Balda, Pepperberg, & Kamil, 1998; Kamil & Roitblat, 1985). Herzog (1990) found that young garter snakes that were briefly exposed to a predator model on seven occasions showed greater tendencies to flee weeks afterward. Snakes, especially generalist species, may encounter seasonal fluctuations in prey availability, abundance, or dispersal patterns, and hibernation results in extensive periods without feeding experience. Snakes that are able to retain their abilities to detect, capture, and efficiently consume prey upon each encounter may suffer less from predation, and may be better able to diversify their diets.

Early feeding experience and survival

Although experience may not affect long-term prey-specific foraging efficiency in these garter snakes, feeding experience appears to play a very important role in the development of foraging skills in young garter snakes. Lind and Welsh (1994) report age-related differences in diet and foraging behavior in wild *Thamnophis atratus*, indicating

that an ontogenetic shift in feeding behavior occurs during early adulthood. The role of feeding experience and learning when undergoing such shifts is in need of further research. In addition to feeding behaviors, snakes are known to undergo ontogenetic shifts in preferred habitat during foraging. Savitsky and Burghardt (2000) found that young water snakes (*Nerodia rhombifer*) forage in highly vegetated areas near shallow water, whereas adult water snakes frequently forage in the open water where predation risk may be higher. Predator pressure may differentially affect habitat selection by snakes of different size classes. Neonatal survival rates may increase through the selection of foraging habitats where predation risks are minimized and by improving foraging skills through learning.

In comparison to snakes, the development of predation in mammals is characterized by long periods of experience, where observational learning, play, social competition, and practice all may facilitate the ontogeny of adult predatory skills (Caro, 1980; Polsky, 1975; Vargas & Anderson, 1998). Precocial species with no maternal care, such as *T. sirtalis*, rely on feeding experience and maturation by physical growth to facilitate the development of foraging skills. Neonatal *T. sirtalis* may often be born into unpredictable and fluctuating environments. For a precocial predatory generalist, it may pay to be relatively unspecialized at birth, and yet have the capacity to develop foraging capacities that are comparable to specialist species (e.g., Mori, 1996) after only a limited amount of feeding experience.

The capacity to rapidly acquire feeding skills may be especially beneficial to a species that is subject to high levels of neonatal predation. Lawton and Hughes (1985) and Brown and Richardson (1987) found that the foraging behavior of muricid

gastropods and predatory crabs is greatly influenced by mortality risks, and that foraging skills are rapidly acquired with feeding experience. Snake survival rates in the wild are difficult to quantify. Mortality seems to be fairly high for wild neonatal *T. sirtalis* (Jayne & Bennett, 1990). Snakes may be especially vulnerable to predation while foraging in areas without cover, underwater, or along water banks. Young *T. sirtalis* moving into such areas may be especially susceptible to predation. Learning and remembering how to rapidly detect, capture, handle, and swallow prey would be beneficial for a species with high neonatal and juvenile mortality due to predation, and would further facilitate the invasion of new habitats and feeding niches.

Heritabilities for physiological, morphological, and behavioral traits associated with anti-predator and foraging behavior by *T. sirtalis* have been widely studied (Arnold, 1981; Arnold & Bennett, 1984; Burghardt & Schwartz, 1999; see Brodie & Garland, 1993, for review). Measuring selection on these traits in the wild is extremely difficult. However, Jayne and Bennett (1990) assayed several important morphological and behavioral traits associated with predator encounters, and determined that some of these served as important predictors of survival in the wild. Thus, there is probably strong selection acting on traits such as scalation patterns, locomotor abilities, striking, reversing direction of travel, and fleeing. Similarly, if there is high predation on foraging *T. sirtalis* neonates, there may be selection for the rapid acquisition of feeding skills. Litter effects were found for capture and total consumption times at FT1 of Experiment I, and Burghardt and Krause (1999) found litter effects for various prey consumption latencies. Further work could examine the relationship between the acquisition of predatory skills and survival in the wild by *T. sirtalis*.

CHAPTER 5

SUMMARY AND CONCLUSION

The foraging behavioral repertoire of *T. sirtalis* consists of a wide array of responses to many prey species. Also, *T. sirtalis* utilizes diverse food sources (Fitch, 1982; Kephart, 1982; Gregory & Nelson, 1991), which may, in part, explain its widespread geographic distribution. I found that learning to detect and consume prey occurs quickly in young *T. sirtalis*. Increasing foraging efficiency with feeding experience may be beneficial for a variety of reasons, including reduced energy expenditure and predation risk (Hughes, 1979). The role of learning in the development of predatory behavior is germane to theoretical and empirical studies of foraging behavior. For example, Hughes (1979) generalized energy maximization models to include variables such as prey recognition time and predator learning. In an empirical study, Burrows and Hughes (1991) observed that the majority of individual variation in the foraging behavior of dogwhelks (*Nucella lapillus*) was accounted for by prior feeding experience. When dogwhelks that normally fed upon barnacles were relocated to a region where mussels were available, these transplanted individuals continued eating the less profitable barnacles. Burrows and Hughes' (1991) findings demonstrate the importance of considering ontogenetic and habitat-specific factors in studies of foraging behavior, as learning and early experience may either constrain or facilitate successful feeding.

I focused on morphological and behavioral traits relevant to foraging in *T. sirtalis*, a species that inhabits a wide range of habitats and feeds on a diversity of prey items. Due to the extreme abundance of garter snakes on Beaver Island, and the diversity of available habitats, the system that I studied was ideal for examining morphological and behavioral

variation. My results indicated that geographic variation of morphological traits can occur among snakes separated by very little distance (see also Brown & Weatherhead, 1999). Results from my behavioral experiments revealed diet-induced changes in chemosensory responses and foraging behavior, but some behaviors were unaffected by feeding experience. In this chapter, I review the hypotheses that I tested, attempt to relate the outcomes of my studies to some broader issues, and make some additional comments about future directions for research on this system.

Growth and morphology

In my field work, I found that differences in available diet are associated with significant differences in body sizes, but only among adult female garter snakes. Originally, I predicted that males from Miller's marsh would also be larger than males from McCafferty farm. The explanation for the lack of difference among males can not be determined with my data, but the site difference in degree of sexual dimorphism raises some interesting questions. Some testable hypotheses include food resource partitioning between males and females, with males from both sites having similar diets, or site differences in relative survival rates among sexes, or sampling error.

Also, as predicted, females from both sites had greater relative head sizes than male snakes. Female biased head size dimorphism among adult *T. sirtalis* has been frequently reported (see King et al., 1999 for review). In contrast, the presence of head size dimorphism in neonates has rarely been reported (see below). For adult snakes, relative head sizes were not substantially correlated with available diet, possibly due to the effects of gene flow between sites. Adult snakes from Miller's marsh had significantly greater inter-ocular distances than snakes from McCafferty farm. However, jaw or head

lengths are the dimensions that are most often measured in studies of diet-induced morphological variation (Forsman, 1996a; Forsman & Lindell, 1991).

In my laboratory studies, I tested several hypotheses on postpartum morphology and the development of morphological differences in snakes reared on different diets. I hypothesized that snakes with fish in their diets would grow more than snakes feeding exclusively on worms. However, diet only had a weak effect on the growth rates of laboratory born neonates through 240 days. Thus, substantial size differences among adult snakes from Miller's marsh and McCafferty farm may not appear until after the first year. Due to body size constraints, the diets of young snakes from both sites may be restricted to worms. Site differences in body sizes may appear when the snakes at Miller's marsh are large enough to consume adult amphibians. I predicted that neonates born to females collected at Miller's marsh would have greater SVLs and body weights, but not head sizes, than neonates born to females from McCafferty farm. However, neonatal SVL and mass did not differ among sites, and neonates born to mothers from Miller's marsh had greater JLs and IODs than neonates born to females from McCafferty farm. With the exception of TL, the neonates from Miller's marsh were slightly larger than the neonates born to females from McCafferty farm (sexes combined) for all measurements. A maternal effect could account for the slightly larger sizes of the neonates born to mothers from Miller's marsh over the neonates born to females from McCafferty farm, as well as the greater JLs and IODs.

As discussed in Chapter 2, the significant head size differences among male and female snakes may be evolutionarily important. Sexual head size dimorphism in neonatal garter snakes has been reported by Shine and Crews (1988), King et al. (1999), and in my

dissertation. However, the ecological or evolutionary consequences of head size dimorphism at birth have not been established for snakes, although some possibilities have been proposed (King et al.; Shine, 1991, 1994; see Chapter 2). Head size dimorphism among the sexes probably has no relationship to courtship and mating in garter snakes, and may simply be a byproduct of growth inhibitory effects of high androgen levels in males. Data on the diets and feeding behaviors of young garter snakes in the wild may help to explain the presence of neonatal head size dimorphism, especially if food resource partitioning or feeding capacity correlate with head size differences. Furthermore, it is likely that some populations of garter snakes will not have sexually dimorphic head sizes at birth (see Arnold & Peterson, 1989), and diet or feeding behavior among young snakes in these populations may not differ.

To date, the work of Grudzien et al. (1992) is the most thorough examination of geographic variation in garter snake head sizes. These authors found that garter snakes inhabiting four of the main islands comprising the Beaver Archipelago (Beaver, Hog, High, and Garden Islands) differed in relative head sizes from mainland garter snakes (Waugoshance Point). Additionally, principal component analyses revealed some differences in head dimensions among island populations. Grudzien et al. did not report descriptive results in their paper and did not control for body size in their analyses, but it appears that snakes from the island populations have greater head sizes than mainland snakes. A related finding comes from King (1989), who reported greater body sizes in island populations of *T. sirtalis* and water snakes (*Nerodia sipedon*) compared to mainland populations. The findings of Grudzien et al. and King (1989) contrast with Case's (1978) claim that snakes from island populations are smaller than snakes from

mainland populations because of reduced food availability. Due to reduced predation and intraspecific competition for food, resource availability may be enhanced in some island populations, such as at Miller's marsh, in comparison to mainland sites.

Variables such as predation, inter-specific competition, and other sources of mortality need to be accounted for as well as dietary differences. Size differences among island and mainland snakes could also be explained by differences in population age-structure. Fecundity and predation pressure may vary between island and mainland populations of snakes, resulting in differences in population age structure. For example, there may be more adult snakes or larger adult snakes in island populations, or neonatal survival may be unequal between island and mainland populations. Forsman (1993) found that predation pressure did not have a strong influence on the population age-structure of adders, although predation may have caused variation in annual survival rates. In a six-year study of survival in a natural population of adders, Forsman (1993) found that survival of large adders was lowest during one year of the study, survival of smaller snakes was lowest during another, and snakes of intermediate sizes had the highest survival rates during another year. No differences between size classes were found during two years of the study. Forsman (1993) suggests that fluctuations in food availability were the primary determinants of differential survival rates between the different size classes. For example, food was scarce during the year that the snakes of intermediate size had higher survival rates than small and large snakes. Forsman (1993) argued that an intermediate size in snakes is optimal when resources are scarce because small snakes, despite requiring less food for maintenance, are less able to capture and consume prey than larger snakes. Furthermore, small snakes are more limited in the size

of prey they can swallow because of their limited mouth-gape size. Large snakes may suffer more than intermediate-sized snakes during periods of food scarcity because, despite the fasting capabilities of larger snakes, their caloric needs are higher than intermediate-sized snakes (Forsman, 1993).

The findings described for the adders may also apply to the garter snakes on Beaver Island. The age-structures of the two sites may differ owing to the differential effects of food scarcity on survival. For example, a dry climate may drastically reduce food availability at McCafferty farm, thereby reducing the numbers of large and small snakes. The snakes at Miller's marsh would experience the same dry climate, but food supplies appear to be much greater at Miller's marsh than at McCafferty farm, even during dry periods. During both field seasons, rainfall was rare and only one snake at McCafferty farm was found with stomach contents. In contrast, the snakes at Miller's marsh consumed many different species of amphibians that were in constant supply due to the availability of a permanent body of water. Thus, my conclusion that dietary differences account for body size variation between the two sites is tentative until alternative explanations, such as differences in population age-structures, are taken into account.

Chemosensory responses to prey

Correlated geographic variation of chemoreceptive responses to locally abundant prey have been reported for *T. sirtalis*, but in populations that are separated by considerable distances (Arnold, 1992). In my study, site was not a significant factor in tests for neonatal chemoreceptive responses to fish and worm extracts, which may be due to the effects of gene flow. The ecological validity of these tests would have been

improved had I used frog stimuli. However, using frogs would have been logistically difficult given the large numbers needed for the long-term goals of the chemoreception, growth, and foraging behavior studies.

Snake chemosensory response profiles generally correlate with natural diets (Arnold, 1992; Burghardt, 1993) and some response tendencies may be retained following speciation. The latter possibility has been shown in the worm specialist, *T. butleri*, which attacks fish extracts and readily consumes fish in captivity, but not in the wild (Burghardt, 1969; Lyman-Henley & Burghardt, 1995). Similarly, captive neonatal *T. sirtalis* from source populations that do not consume fish will attack fish extracts and consume fish in captivity (Arnold, 1992; Burghardt et al., 2000; this study). Developmental changes in chemosensory responses due to maturation or feeding experience have been reported, and these response changes are often restricted to a single type of prey (Burghardt, 1993; Lyman-Henley & Burghardt, 1995; Mushinsky & Lotz, 1980). Garter snakes reared on fish show enhanced chemoreceptive responses to fish extract, even if the snakes come from populations that do not eat fish. Further work is needed to determine whether chemosensory response changes due to feeding experience relate to ecological differences among populations. Comparisons between fish-eating and non-fish-eating populations of *T. sirtalis* are needed. Such comparisons could serve to determine whether enhanced chemoreceptive responses to fish facilitate the incorporation of fish into the snakes' natural diets.

In my work, I found some evidence for diet-induced changes in chemosensory responses to prey. However, in my sample, diet did not appear to have long-term effects on the snakes' relative responses to fish or worms, although differences between neonatal

and adult chemosensory responses to prey have been reported for water snakes *Nerodia sipedon* (Gove & Burghardt, 1975) and garter snakes (Greenwell et al., 1984). If chemosensory responses to prey are over-enhanced as a result of feeding experience, then fluctuations in prey abundance could result in decreased chances of prey detection. If chemosensory responses are over-inhibited due to feeding experience, then prey detection could be hindered. Ideally, in addition to studying neonates, the natural diets and chemosensory responses of wild-caught adults could be studied.

Ontogeny of feeding skills

As hypothesized, the development of predatory skills in neonates was affected by feeding experience, but feeding skills by wild-caught adults appeared to be more affected by maturation, or prey type, than prior feeding experience, although individual feeding histories for wild-caught adults were not available. Thus, the potential for behavioral and morphological plasticity in *T. sirtalis* may be greater during early ontogeny than in adulthood. In Experiment I, total prey consumption times decreased considerably after the snakes' first 11 or 12 feedings. My prediction that feeding skills would asymptote, as measured by latencies to consume prey after 11 or 12 additional feedings, was confirmed. Based on the results of Burghardt and Krause (1999), I predicted that feeding on pure diets of fish or worms would results in greater decreases in approach and consumption times in comparison to snakes reared on mixed diets. The snakes in the mixed diet group did not significantly decrease their total worm consumption times after feeding experience, which is consistent with Burghardt and Krause's (1999) findings. However, the mean worm consumption time by the mixed diet group was initially lower than for the snakes in the pure diet group.

Additional dietary effects on early feeding skills were evident during the diet-switching phase of Experiment I. Feeding strictly on worms during early development interfered with the snakes' abilities to switch to fish, whereas feeding on fish facilitated the snakes' abilities to feed on worms. Interference and facilitation effects may only be found in young garter snakes, as wild-caught, adult snakes from McCafferty farm were as proficient at feeding on frogs and fish as adults from Miller's marsh.

I originally predicted that the adult snakes from both sites would approach worms at the same rate. However, the snakes from McCafferty farm approached worms (and fish) more rapidly than the snakes from Miller's marsh, possibly due to motivational effects (e.g., hunger). I attempted to standardize hunger levels as much as possible, but the dry weather may have resulted in a prolonged reduction of worm availability for the snakes from McCafferty farm. The snakes did not significantly differ in their total times to consume worms, fish, or frogs, which suggests that feeding experience does not have long-term, prey-dependent effects on adult garter snake feeding skills. In Chapter 4, I suggested the possibility that snake maturation (e.g., increased body size) may be a better determinant of prey consumption abilities than feeding experience (see Arnold, 1993 for review). Thus, maturation may explain why adults from McCafferty farm consumed frogs as proficiently as the snakes from Miller's marsh. Additionally, the antipredator behavior or morphology of certain types of prey (e.g., frogs) may limit the degree to which feeding proficiency can increase with predator experience. It is also possible that the snakes' individual prey preferences, and not prey foraging abilities, influenced the outcome of Experiment III.

Phenotypic plasticity and learning

Learning can be conceptualized as a suite of phenotypes produced by a single genotype. Thus, learning is a type of phenotypic plasticity. Conceptually, learning has not been fully integrated with the concept of phenotypic plasticity, although progress in this endeavor is being made (reviewed in Pigliucci, in press). Population differences in the learning capacities of several species have been identified, which indicates strong selection for the development of behavioral flexibility in some populations, and not in others (Foster, 1999). Furthermore, the conceptual integration of learning and phenotypic plasticity requires a clear definition of learning (see Domjan, 1998), as behavioral plasticity may be present in the absence of learning (Stirling & Roff, 2000). One challenge is to identify what distinguishes learning from other types of plasticity.

Behavioral plasticity is often regarded as a distinct type of phenotypic plasticity, primarily because behavioral responses to the environment often take place immediately, and are more likely to be reversible than morphological traits (Cavalli-Sforza 1974; West-Eberhard, 1989). Thus, learning is generally regarded as a reversible and flexible process, although constraints on learning are well known, and learning may be adaptively specialized within a specific behavioral system, such as sexual behavior (Domjan & Hollis, 1988; Sevenster, 1973). However, the capacity for reversibility does not clearly distinguish morphological plasticity and learning. Byers (1998) stated that "Phenotypic plasticity in systems that support strength and endurance is adaptive" (p. 205), and primarily applied his statement to vertebrate muscular and skeletal plasticity. Byers' statement implies that morphological changes in response to environmental circumstances can be reversed, as strength and endurance levels can atrophy (see also

Dodson, 1989). Day and McPhail's (1996) study of morphological and behavioral plasticity in stickleback foraging behavior suggests that the immediacy of the changes that characterize learning, rather than its reversibility, distinguish behavioral plasticity from morphological plasticity.

The need for immediate, flexible responses to environmental changes may relate to the type(s) of environments inhabited. Komers (1997) stated that "The plasticity of behavior consists of an array of behavioural responses to varying environmental conditions" (p. 161). Consistent with earlier predictions (e.g., Bradshaw, 1965; Morse, 1980), Komers (1997) concluded that, generally, environmental and behavioral variability are positively correlated (see also Carroll & Corneli, 1999). In terms of selection, animals that make immediate and reversible behavioral responses to environmental changes may be at a reproductive advantage over animals that do not make immediate and reversible behavioral responses. Thus, selection favors behavioral flexibility in fluctuating environments (Komers, 1997).

Phenotypic plasticity is known to buffer the effects of natural selection (Schlichting & Pigliucci, 1998; Stearns, 1989), thereby enabling species such as *T. sirtalis* to invade new habitats and survive environmental fluctuations. Thompson (1991) maintained that plasticity is more likely to contribute to fitness in a heterogeneous environment when 'physiological buffering' to poor conditions and improved responses to favorable conditions can both occur. 'Behavioral buffering' could be included here too, because how organisms adjust to poor conditions may include migration or food switching. Physiological, neural, and behavioral plasticity may all contribute to survival when conditions become favorable or poor. In relation to my dissertation, an improved

response to a favorable condition may include invading a new feeding niche. The evolution of generalist strategies may be more probable under fluctuating conditions (Thompson, 1991). Learning is one behavioral mechanism that may allow organisms to adjust to poor conditions and capitalize on favorable conditions.

Studies of geographic variation in behavior have demonstrated population differences in learning capacity. For example, Huntingford, Wright, & Tierney (1994) found that population differences in anti-predator behavior of three-spine sticklebacks were based on learning. In addition to population differences, individual differences in learning capacity are evolutionarily important (Gotceitas & Colgan, 1988). Werner, Mittelbach, & Hall (1981) discovered that individual differences in learning capacity facilitate changes in food habitat specializations in bluegill sunfish (*Lepomis macrochirus*). Individuals that are able to learn more rapidly than others may be better able to invade novel food sources or switch to more profitable ones (see also Gotceitas & Colgan, 1988). Individual (or population) differences in diet and foraging behavior in wild garter snakes may be related to variation in levels of behavioral plasticity. Genetic differences are known to account for population variation in garter snake morphology (Burghardt & Schwartz, 1999), chemosensory responses (Arnold, 1981a,b,c; Burghardt & Schwartz, 1999), and prey handling behaviors (Drummond & Burghardt, 1983; Garcia & Drummond, 1990). In addition, learning capabilities may influence prey choice within populations, explain differences among populations, and interact with diet, morphology, sex, and reproductive fitness (Burghardt et al., 2000; Ehlinger, 1999; Gotceitas & Colgan, 1988).

LITERATURE CITED

Aickin, M., & Gensler, H. (1996). Adjusting for multiple testing when reporting research results: The Bonferroni vs Holm methods. American Journal of Public Health, 86, 726-728.

Anderson, J. R. (1991). Is human cognition adaptive? Behavioral and Brain Sciences, 14, 471-517.

Anderson, J. R., & Schooler, L. J. (1991). Reflections of the environment in memory. Psychological Science, 2, 396-408.

Arnold, S. J. (1978). Some effects of early experience on feeding responses in the common garter snake, *Thamnophis sirtalis*. Animal Behaviour, 26, 455-462.

Arnold, S. J. (1981a). Behavioral variation in natural populations. I. Phenotypic, genetic, and environmental correlations between chemoreceptive responses to prey in the garter snake, *Thamnophis elegans*. Evolution, 35, 489-509.

Arnold, S. J. (1981b). The microevolution of feeding behavior. In A. Kamil, & T. Sargent (Eds.), Foraging behavior: Ecological, ethological and psychological approaches (pp. 409-453). New York: Garland Press.

Arnold, S. J. (1981c). Behavioral variation in natural populations. II. The inheritance of a feeding response in crosses between geographic races of the garter snake, *Thamnophis elegans*. Evolution, 35, 510-515.

Arnold, S. J. (1992). Behavioural variation in natural populations. VI. Prey responses by two species of garter snakes in three regions of sympatry. Animal Behaviour, 44, 705-719.

Arnold, S. J. (1993). Foraging theory and prey-size-predator-size relations in snakes. In R. A. Seigel & J. T. Collins (Eds.), Snakes: Ecology and Behavior (pp. 87-115). New York: McGraw-Hill.

Arnold, S. J., & Bennett, A. F. (1984). Behavioural variation in natural populations, III. Antipredator displays in the garter snake, *Thamnophis radix*. Animal Behaviour, 32, 1108-1118.

Arnold, S. J., & Peterson, C. R. (1989). A test for temperature effects on the ontogeny of shape in the garter snake *Thamnophis sirtalis*. Physiological Zoology, 62, 1316-1333.

Balda, R. P., Pepperberg, I. M., & Kamil, A. C. (Eds.) (1998). Animal cognition in nature. San Diego: Academic Press.

Bradshaw, A. D. (1965). Evolutionary significance of phenotypic plasticity in plants. Advances in Genetics, 13, 115-155.

Brodie, E. D., & Garland, T. (1993). Quantitative genetics of snake populations. In R. A. Siegel, & J. T. Collins (Eds.) Snakes: Ecology and behavior (pp. 315-362). New York: McGraw-Hill, Inc.

Brodie, E. D., & Russell, N. H. (1999). The consistency of individual differences in behaviour: Temperature effects on antipredator behaviour in garter snakes. Animal Behaviour, 57, 445-451.

Brown, G. P., & Weatherhead, P. J. (1999). Female distribution affects mate searching and sexual selection in male northern water snakes (*Nerodia sipedon*). Behavioral Ecology and Sociobiology, 47, 9-16.

Brown, K. M., & Richardson, T. D. (1987). Foraging ecology of the southern oyster drill *Thais haemastoma* (Gray): Constraints on prey choice. Journal of Experimental Marine Biology and Ecology, 114, 123-141.

Burghardt, G. M. (1966). Stimulus control of the prey attack response in naïve garter snakes. Psychonomic Science, 4, 37-38.

Burghardt, G. M. (1967). Comparative prey-attack studies of newborn snakes of the genus *Thamnophis*. Science, 157, 718-721.

Burghardt, G. M. (1969). Comparative prey-attack studies in newborn snakes of the genus *Thamnophis*. Behaviour, 33, 77-114.

Burghardt, G. M. (1970a). Chemical perception in reptiles. In J. W. Johnston, Jr., D. G. Moulton, & A. Turk, (Eds.), Communication by chemical signals (pp. 241-308). New York: Appleton-Century-Crofts.

Burghardt, G. M. (1970b). Intraspecific geographical variation in chemical food cue preference of newborn garter snakes (*Thamnophis sirtalis*). Behaviour, 36, 246-257.

Burghardt, G. M. (1975). Chemical prey preference polymorphism in newborn garter snakes, *Thamnophis sirtalis*. Behaviour, 52, 202-225.

Burghardt, G. M. (1977). Learning processes in reptiles. In C. Gans, & D. Tinkle (Eds.), The biology of the reptilia: Vol. 7. Ecology and behavior (pp. 555-681). New York: Academic Press.

Burghardt, G. M. (1978). Behavioral ontogeny in reptiles: Whence, whither, and why? In G. M. Burghardt, & M. Bekoff (Eds.), The development of behavior: Comparative and evolutionary aspects (pp. 149-174). New York: Garland STPM Press.

Burghardt, G. M. (1990). Chemically mediated predation in vertebrates: Diversity, ontogeny, and information. In D. McDonald, D. Muller-Schwarze, & S. Natynczuk (Eds.), Chemical signals in vertebrates, vol. 5 (pp. 477-499). Oxford: Oxford University Press.

Burghardt, G. M. (1992). Prior exposure to prey cues influences chemical prey preference and prey choice in neonatal garter snakes. Animal Behaviour, 44, 787-789.

Burghardt, G. M. (1993). The comparative imperative: Genetics and ontogeny of chemoreceptive prey responses in natricine snakes. Brain, Behavior, and Evolution, 41, 138-146.

Burghardt, G. M., & Denny, D. (1983). Effects of prey movement and prey odor on feeding in garter snakes. Zeitschrift für Tierpsychologie, 62, 329-347.

Burghardt, G. M., & Krause, M. A. (1999). Plasticity of foraging behavior in garter snakes (*Thamnophis sirtalis*) reared on different diets. Journal of Comparative Psychology, 113, 277-285.

Burghardt, G. M., & Schwartz, J. M. (1999). Geographic variations on methodological themes in comparative ethology: A natricine snake perspective. In S. A. Foster, & J. A. Endler (Eds.), Geographic variation in behavior: Perspectives on evolutionary mechanisms (pp. 69-94). Oxford: Oxford University Press.

Burghardt, G. M., Layne, D. G., & Konigsberg, L. (2000). The genetics of dietary experience in a restricted natural population, Psychological Science, 11, 69-72.

Burghardt, G. M., Wilcoxon, H. C., & Czaplicki, J. A. (1973). Conditioning in garter snakes: Aversion to palatable prey induced by delayed illness. Animal Learning and Behavior, 1, 317-320.

Burrows, M. T., & Hughes, R. N. (1991). Variation in foraging behaviour among individuals and populations of dogwhelks, *Nucella lapillus*: Natural constraints on energy intake. Journal of Animal Ecology, 60, 497-514.

Byers, J. A. (1998). Biological effects on locomotor play: Getting into shape, or something more specific. In M. Bekoff & J. A. Byers (Eds.), Play behavior in animals (pp. 205-220). Cambridge: Cambridge University Press.

Caro, T. M. (1980). Effects of the mother, object play, and adult experience on predation in cats. Behavioral and Neural Biology, 29, 29-51.

Carroll, S. P., & Corneli, P. S. (1999). The evolution of behavioral norms of reaction as a problem in ecological genetics. In S. A. Foster, & J. A. Endler (Eds.), Geographic variation in behavior: Perspectives on evolutionary mechanisms (pp. 52-68). Oxford: Oxford University Press.

Carpenter, C. C. (1952). Comparative ecology of the common garter snake (*Thamnophis sirtalis*), the ribbon snake (*Thamnophis s. sauritus*), and Butler's garter snake (*Thamnophis butleri*) in mixed populations. Ecological Monographs, 22, 235-258.

Case, T. J. (1978). A general explanation for insular body size trends in terrestrial vertebrates. Ecology, 59, 1-18.

Cavalli-Sforza, L.L. (1974). The role of plasticity in biological and cultural evolution. Annals of the New York Academy of Sciences, 231, 43-59.

Conant, R., & Collins, J. T. (1991). A field guide to reptiles and amphibians of eastern and central North America (3rd ed.). Boston: Houghton Mifflin.

Cooper, W. E., Jr. (1998). Evaluation of swab and related tests as a bioassay for assessing responses by squamate reptiles to chemical stimuli. Journal of Chemical Ecology, 24, 841-866.

Cooper, W. E., Jr., & Burghardt, G. M. (1990). A comparative analysis of scoring methods for chemical discrimination of prey by squamate reptiles. Journal of Chemical Ecology, 16, 45-65.

Crews, D., Diamond, M., Whittier, J., & Mason, R. (1985). Small male body size in garter snakes depends on testes. American Journal of Physiology, 249, R62-R66.

Croy, M. I., & Hughes, R. N. (1991a). The influence of hunger on feeding behaviour and on the acquisition of learned foraging skills by the fifteen-spined stickleback, *Spinachia spinachia* L. Animal Behaviour, 41, 161-171.

Croy, M. I., & Hughes, R. N. (1991b). The role of learning and memory in the feeding behaviour of the fifteen-spined stickleback, *Spinachia spinachia* L. Animal Behaviour, 41, 149-159.

Cunningham, P. N., & Hughes, R. N. (1984). Learning of predatory skills by shorecrabs, *Carcinus maenas*, feeding on mussels and dogwhelks. Marine Ecology Program Series, 16, 21-26.

Curio, E. (1976). The ethology of predation. Berlin: Springer-Verlag.

Cuthill, I. C., Kacelnik, A., Krebs, J. R., Haccou, P, & Iwasa, Y. (1990). Starlings exploiting patches: The effect of recent experience on foraging decisions. Animal Behaviour, 40, 635-640.

Day, L. B., Crews, D., & Wilczynski, W. (1999). Spatial and reversal learning in congeneric lizards with different foraging strategies. Animal Behaviour, 57, 393-407.

Day, T., & McPhail, J. D. (1996) The effect of behavioural and morphological plasticity on foraging efficiency in the threespine stickleback (*Gasterosteus* sp.). Oecologia, 108, 380-388.

Dickinson, J. A. (1979). The effects of artificial cover availability on the ecology and movements of a population of the garter snake, *Thamnophis s. sirtalis*. Unpublished Master's Thesis, Central Michigan University.

Dix, M. W. (1968). Snake food preference: Innate intraspecific geographical variation. Science, 159, 1478-1479.

Dodson, S. (1989). Predator-induced reaction norms: Cyclic changes in shape and size can be protective. Bioscience, 39, 447-452.

Domjan, M. (1998). The principles of learning and behavior (4th ed.). Pacific Grove, CA: Brooks/Cole Publishing Company.

Domjan, M., & Hollis, K. L. (1988). Reproductive behavior: A potential model system for adaptive specializations in learning. In R. C. Bolles, & M. D. Beecher (Eds.), Evolution and learning (pp. 213-237). Hillsdale, NJ: Erlbaum.

Drummond, H. (1983). Aquatic foraging in garter snakes: A comparison of specialists and generalists. Behaviour, 86, 1-30.

Drummond, H., & Burghardt, G. M. (1983). Geographic variation in the foraging behavior of the garter snake (*Thamnophis elegans*). Behavioral Ecology and Sociobiology, 12, 43-48.

Ehlinger, T. J. (1999). Ecology, phenotype, and character evolution in bluegill sunfish. In S. A. Foster, & J. A. Endler (Eds.), Geographic variation in behavior:

Perspectives on evolutionary mechanisms (pp. 121-138). Oxford: Oxford University Press.

Fitch, H. S. (1965). An ecological study of the garter snake, *Thamnophis sirtalis*. University of Kansas Publications, Museum of Natural History, 15, 493-564.

Fitch, H. S. (1981). Sexual size differences in reptiles. University of Kansas Publications of the Museum of Natural History, 62, 1-53.

Fitch, H. S. (1982). Resources of a snake community in prairie-woodland habitat in northeastern Kansas. In N. J. Scott (Ed.), Herpetological communities. Washington, D. C.: U. S. Fish and Wildlife Services.

Fitch, H. S. (1987). Collecting and life history techniques. In R. A. Seigel, J. T. Collins, & S. S. Novak (Eds.), Snakes: Ecology and evolutionary biology (pp. 143-164). New York: Macmillan Publishing Company.

Ford, N. B., & Burghardt, G. M. (1993). Perceptual mechanisms and the behavioral ecology of snakes. In R. A. Siegel, & J. T. Collins (Eds.), Snakes: Ecology and behavior (pp. 117-164). New York: McGraw-Hill, Inc.

Ford, N. B., & Siegel, R. A. (1989). Phenotypic plasticity in reproductive traits: Evidence from a viviparous snake. Ecology, 70, 1768-1774.

Forsman, A. (1991). Adaptive variation in head size in *Vipera berus* L. populations. Biological Journal of the Linnean Society, 43, 281-296.

Forsman, A. (1994). Growth rate and survival in relation to relative head size in *Vipera berus*. Journal of Herpetology, 28, 231-238.

Forsman, A. (1996a). An experimental test for food effects on head size allometry in juvenile snakes. Evolution, 50, 2536-2542.

Forsman, A. (1996b). Body size and net energy gain in gape-limited predators: A model. Journal of Herpetology, 30, 307-319.

Forsman, A., & Lindell, L. E. (1991). Trade-off between growth and energy storage in male *Vipera berus* (L.) under different prey densities. Functional Ecology, 5, 717-723.

Forsman, A., & Lindell, L. E. (1993). The advantage of a big head: Swallowing performance in adders, *Vipera berus*. Functional Ecology, 7, 183-189.

Foster, S. A. (1999). The geography of behaviour: An evolutionary perspective. Trends in Ecology and Evolution, 14, 190-195.

Foster, S. A., & Endler, J. A. (Eds.) (1999). Geographic variation in behavior: Perspectives on evolutionary mechanisms. Oxford: Oxford University Press.

Fuchs, J. L., & Burghardt, G. M. (1971). Effects of early experience on the responses of garter snakes to food chemicals. Learning and Motivation, 2, 271-279.

Garcia, C. M., & Drummond, H. (1990). Population differences in fish-capturing ability of the Mexican aquatic garter snake (*Thamnophis melanogaster*). Journal of Herpetology, 24, 412-416.

Gibbons, J. W., & Lovich, J. E. (1990). Sexual dimorphism in turtles with an emphasis on the slider turtle (*Trachemys scripta*), Herpetological Monographs, 4, 1-29.

Gilchrist, G. W. (1995). Specialists and generalists in changing environments. I. Fitness landscapes of thermal sensitivity. American Naturalist, 146, 252-270.

Gillingham, J. C. (1988). The amphibians and reptiles of Beaver Island. The Journal of Beaver Island History, 3, 87-115.

Gillingham, J. C., Rowe, J., & Weins, M. A. (1990). Chemosensory orientation and earthworm location by foraging eastern garter snakes (*Thamnophis s. sirtalis*). In D. W. MacDonald, D. Muller-Schwarze, & S. E. Natynczuk (Eds.), Chemical signals in vertebrates, Vol. 5 (pp. 522-532). Oxford: Oxford University Press.

Gotceitas, V., & Colgan, P. (1988). Individual variation in learning by foraging bluegill sunfish (*Lepomis macrochirus*). Journal of Comparative Psychology, 102, 294-299.

Gove, D., & Burghardt, G. M. (1975). Responses of ecologically dissimilar populations of the water snake *Natrix s. sipedon* to chemical cues from prey. Journal of Chemical Ecology, 1, 25-40.

Graves, B. M., Halpern, M., & Gillingham, J. C. (1993). Effects of vomeronasal deafferentation on home range use in a natural population of eastern garter snakes, *Thamnophis sirtalis*. Animal Behaviour, 45, 301-311.

Greenwell, M. G., Hall, M., & Sexton, O. J. (1984). Phenotypic basis for a feeding change in an insular population of garter snakes. Developmental Psychobiology, 17, 457-463.

Gregory, P. T., & Larsen, K. W. (1993). Geographic variation in reproductive characteristics among Canadian populations of the common garter *snake (Thamnophis sirtalis*. Copeia, 1993, 946-958.

Gregory, P. T., & Nelson, K. J. (1991). Predation on fish and intersite variation in the diet of common garter snakes, *Thamnophis sirtalis*, on Vancouver Island. Canadian Journal of Zoology, 69, 988-994.

Gregory, P. T., & Prelypchan, C. J. (1994). Analysis of variance of first-year growth in captive garter snakes (*Thamnophis elegans*) by family and sex. Journal of Zoology (London) 232, 313-322.

Grudzien, T. A., Huebner, B. J., Cvetkovic, A., & Joswiak, G. R. (1992). Multivariate analysis of head shape in *Thamnophis s. sirtalis* (Serpentes: Colubridae) among island and mainland populations from northeastern Lake Michigan. American Midland Naturalist, 127, 339-347.

Halloy, M., & Burghardt, G. M. (1990). Ontogeny of fish capture and ingestion in four species of garter snakes (*Thamnophis*). Behaviour, 112, 299-318.

Halpern, M. (1987). The organization and function of the vomeronasal system. Annual Review of Neuroscience, 10, 325-362.

Halpern, M. (1992). Nasal chemical senses in reptiles: Structure and function. In. C Gans & D. Crews (Eds.), Biology of the reptilia: Hormones, brain, and behavior (Vol. 18, Physiology E) (pp. 423-523). Chicago: The University of Chicago Press.

Herzog, H. A., Jr. (1990). Experiential modification of defensive behaviors in garter snakes, *Thamnophis sirtalis*. Journal of Comparative Psychology, 104, 334-339.

Holtzmann, D. A., Harris, T. W., Aranguren, G., & Bostock, E. (1999). Spatial learning of an escape task by young corn snakes, *Elaphe guttata guttata*. Animal Behaviour, 57, 51-60.

Hughes, R. N. (1979). Optimal diets under the energy maximization premise: The effects of recognition time and learning. The American Naturalist, 113, 209-221.

Hughes, R. N., & Blight, C. M. (1999). Algorithmic behaviour and spatial memory are used by two intertidal fish species to solve the radial maze. Animal Behaviour, 58, 601-613.

Huntingford, F. A., Wright, P. J., & Tierney, J. F. (1994). Adaptive variation in antipredator behaviour in threespine stickleback. In M. A. Bell & S. A. Foster (Eds.), The evolutionary biology of the threespine stickleback (pp. 277-296). Oxford University Press.

Jayne, B. C., & Bennett, A. F. (1990). Selection on locomotor performance capacity in a natural population of garter snakes. Evolution, 44, 1204-1229.

Kamil, A. C., & Roitblat, H. L. (1985). The ecology of foraging behavior: Implications for animal learning and memory. Annual Review of Psychology, 36, 141-169.

Kause, A., Haukioja, E., & Hanhimaki, S. (1999). Phenotypic plasticity in foraging behavior of sawfly larvae. Ecology, 80, 1230-1241.

Kephart, D. G. (1982). Microgeographic variation in the diets of garter snakes. Oecologica, 52, 287-291.

King, R. B. (1997). Variation in brown snake (*Storeria dekayi*) morphology and scalation: Sex, family, and microgeographic differences. Journal of Herpetology, 31, 335-346.

King, R. B. (1989). Body size variation among island and mainland snake populations. Herpetologica, 45, 84-88.

King, R. B., Bittner, T. D., Queral-Regil, A., & Cline, J. H. (1999). Sexual size dimorphism in neonate and adult snakes. Journal of Zoology (London), 247, 19-28.

King, R. B., Milstead, W. B., Gibbs, H. L., Prosser, M. R., Burghardt, G. M., & McCracken, G. F. (in review). Microsatellite DNA markers reveal multiple paternity and allow discrimination of maternal and genetic effects on garter snake morphology and behavior.

Komers, P. E. (1997). Behavioural plasticity in variable environments. Canadian Journal of Zoology, 75, 161-169.

Krebs, J. R., & Inman, A. J. (1994). Learning and foraging: Individuals, groups, and populations. In L. A. Real (Ed.), Behavioral mechanisms in evolutionary ecology (pp. 46-65). Chicago: University of Chicago Press.

Kuo, Z. Y. (1930). The genesis of the cat's response to the rat. Journal of Comparative Psychology, 11, 1-35.

Kuo, Z. Y. (1938). Further study on the behavior of the cat towards the rat. Journal of Comparative Psychology, 25, 1-8.

Kuo, Z. Y. (1967). The dynamics of behavior development. New York: Plenum Press.

Lanyon, L. E., & Rubin, C. T. (1985). Functional adaptation in skeletal structures. In M. Hildebrand, D. M. Bramble, K. F. Liem, & D. B. Wake (Eds.), Functional vertebrate morphology (pp. 1-25). Cambridge, MA: Harvard University Press.

Lawton, P. & Hughes, R. N. (1985). Foraging behaviour of the crab *Cancer pagurus* feeding on gastropods *Nucella lapillus* and *Littorina littorea*: Comparison with optimal foraging theory. Marine Ecology Progress Series, 27, 143-154.

Leyhausen, P. (1973). On the function of the relative hierarchy of moods (as exemplified by the phylogenetic and ontogenetic development of prey-catching in

carnivores). In K. Lorenz, & P. Leyhausen (Eds.), Motivation of human and animal behavior (pp. 144-247). London: Von Nostrand Reinhold.

Lind, A. J., & Welsh, H. H. (1994). Ontogenetic changes in foraging behavior and habitat use by the Oregon garter snake, *Thamnophis atratus hydrophilus*. Animal Behaviour, 48, 1261-1273.

Losos, J. B., Creer, D. A., Glossip, D., Goellner, R., Hampton, A., Roberts, G., Haskell, N., Taylor, P., & Etling, J. (2000). Evolutionary implications of phenotypic plasticity in the hindlimb of the lizard *Anolis sagrei*. Evolution, 54, 301-305.

Lyman-Henley, L. P., & Burghardt, G. M. (1995). Diet, litter, and sex effects on chemical prey preference, growth, and site selection in two sympatric species of *Thamnophis*. Herpetological Monographs, 9, 140-160.

Mackney, P. A., & Hughes, R. N. (1995). Foraging behaviour and memory in sticklebacks. Behaviour, 132, 1241-1253.

Madsen, T., & Shine, R. (1993). Phenotypic plasticity in body sizes and sexual size dimorphism in European grass snakes. Evolution, 47, 321-325.

Meers, M. B. (in prep.). Practical crocodilian anatomy and its relevance to captive management. In K. Vliet & A. Odum (Eds.), Crocodilian biology and captive management. Silver Springs, MD: American Zoo and Aquarium Association.

Milinski, M., & Heller, R. (1978). Influence of a predator on the optimal foraging behaviour of sticklebacks (*Gasterosteus aculeatus* L.). Nature, 275, 642-644.

Mori, A. (1994). Prey-handling behaviour of newly hatched snakes in two species of the genus *Elaphe* with comparison to adult behaviour. Ethology, 97, 198-214.

Mori, A. (1996). A comparative study of the development of prey-handling behavior in young rat snakes, *Elaphe quadrivirgata* and *E. climacophora*. Herpetologica, 52, 313-322.

Morse, D. H. (1980). Behavioural mechanisms in ecology. Cambridge, MA: Harvard University Press.

Mushinsky, H. R., & Lotz, K. H. (1980). Chemoreceptive responses of two sympatric water snakes to extracts of commonly ingested prey species. Journal of Chemical Ecology 6, 523-535.

Nelson, K. J., & Gregory, P. T. (2000). Activity patterns of garter snakes, *Thamnophis sirtalis*, in relation to weather conditions at a fish hatchery on Vancouver Island, British Columbia. Journal of Herpetology, 34, 32-40.

O'Brien, R. G., & Kaiser, M. K. (1985). MANOVA method for analyzing repeated measures designs: An extensive primer. Psychological Bulletin, 97, 316-333.

Pigliucci, M. (in press). Beyond nature vs. nurture: The genetics, ecology, and evolution of genotype-environment interactions. Baltimore, MD: Johns Hopkins University Press.

Pigliucci, M., Schlichting, C. D., Jones, C. S., & Schwenk, K. (1996). Developmental reaction norms: The interactions among allometry, ontogeny, and plasticity. Plant Species Biology, 11, 69-85.

Polsky, R. H. (1975). Developmental factors in mammalian predation. Behavioral Biology, 15, 353-382.

Polsky, R. H. (1977). The ontogeny of predatory behavior in the golden hamster: I. Influence of age and experience. Behaviour, 61, 25-57.

Queral-Regil, A., & King, R. B. (1998). Evidence for phenotypic plasticity in snake body size and relative head dimensions in response to amount and size of prey. Copeia, 1998, 423-429.

Rivas, J. A., & Burghardt, G. M. (submitted). Understanding sexual size dimorphism in snakes: Wearing the snake's shoes. Manuscript submitted for publication.

Robinson, B. W., & Wilson, D. S. (1995) Experimentally induced morphological diversity in Trinidadian Guppies (*Poecilia reticulata*). Copeia, 1995, 294-305.

Rossman, D. A., Ford, N. B., & Seigel, R. A. (1996). The garter snakes: Evolution and ecology. Norman, Oklahoma: University of Oklahoma Press.

Savitsky, B. A., & Burghardt, G. M. (2000). Ontogeny of predatory behavior in the aquatic specialist snake, *Nerodia rhombifer*, during the first year of life. Herpetological Monographs, 14, 401-419.

Schlichting, C. D., & Pigliucci, M. (1998). Phenotypic evolution: A reaction norm perspective. Sunderland, MA: Sinauer Associates.

Schuett, G., W., & Gillingham, J. C. (1989). Male-male agonistic behavior of the copperhead (*Agkistrodon contortrix*). Amphibia-Reptilia, 10, 243-266.

Scudder-Davis, R. M., & Burghardt, G. M. (1987). Diet and growth in juveniles of the garter snakes *Thamnophis sirtalis infernalis* and *Thamnophis radix radix*. Growth, 51, 74-85.

Serra, G., Chelazzi, G., & Castilla, J. C. (1997). Effects of experience and risk of predation on the foraging behaviour of the South-eastern Pacific muricid *Concholepas concholepas* (Mollusca: Gastropoda). Journal of Animal Ecology, 66, 876-883.

Sevenster, P. (1973). Incompatibility of response and reward. In R. A. Hinde & J. Stevenson-Hinde (Eds.), Constraints on learning (pp. 265-283). London: Academic Press.

Shettleworth, S. J. (1993). Varieties of learning and memory in animals. Journal of Experimental Psychology, 19, 5-14.

Shettleworth, S. J. (1998). Cognition, Evolution, and Behavior. New York: Oxford University Press.

Shine, R. (1986). Sexual differences in morphology and niche utilization in an aquatic snake, *Acrochordus arafurae*. Oecologia, 69, 260-267.

Shine, R. (1990). Proximate determinants of sexual differences in adult body size. American Naturalist, 135, 278-283.

Shine, R. (1991). Why do larger snakes eat larger prey items? Functional Ecology, 5, 493-502.

Shine, R. (1993). Sexual dimorphism in snakes. In R. A. Siegel, & J. T. Collins (Eds.) Snakes: Ecology and behavior (pp. 49-86). New York: McGraw-Hill.

Shine, R. (1994). Sexual size dimorphism in snakes revisited. Copeia, 1994, 326-346.

Shine, R., & Crews, D. (1988). Why male garter snakes have small heads: The evolution and endocrine control of sexual dimorphism. Evolution, 42, 1105-1110.

Sih, A. (1993). Effects of ecological interactions on forager diets: Competition, predation risk, parasitism and prey behavior. In R. N. Hughes (Ed.), Diet selection: An interdisciplinary approach to foraging behaviour (pp. 182-211). Oxford: Blackwell Science.

Stearns, S. C. (1989) The evolutionary significance of phenotypic plasticity. Bioscience 39, 436-445.

Stephens, D. W., & Krebs, J. R. (1986). Foraging theory. Princeton: Princeton University Press.

Stirling, G., & Roff, D. A. (2000). Behaviour plasticity without learning: Phenotypic and genetic variation of naive *Daphnia* in an ecological trade-off. Animal Behaviour, 59, 929-941.

Terrick, T. D., Mumme, R. L., & Burghardt, G. M. (1995). Aposematic coloration enhances chemosensory recognition of noxious prey in the garter snake *Thamnophis radix*. Animal Behaviour, 49, 857-866.

Thompson, J. D. (1991). Phenotypic plasticity as a component of evolutionary change. Trends in Ecology and Evolution, 6, 246-249.

Valone, T. J. (1992). Patch estimation via memory windows and the effects of travel time. Journal of Theoretical Biology, 157, 243-251.

Vargas, A., & Anderson, S. H. (1998). Ontogeny of black-footed ferret predatory behavior towards prairie dogs. Canadian Journal of Zoology, 76, 1696-1704.

Walls, S. C., Belanger, S. S., & Blaustein, A. R. (1993). Morphological variation in a larval salamander: Dietary induction of plasticity of head shape. Oecologia, 96, 162-168.

Waters, R. M. (2000). Feeding behavior of crayfish snakes (*Regina*): Allometry, ontogeny and adaptations to an extremely specialized diet. Unpublished doctoral dissertation, University of Tennessee, Knoxville.

Wcislo, W. T. (1989). Behavioral environments and evolutionary change. Annual Review of Ecology and Systematics, 20, 137-169.

Werner, E., Mittelbach, G. G., & Hall, D. J. (1981). The role of foraging profitability and experience in habitat use by the bluegill sunfish. Ecology, 82, 116-125.

West-Eberhard, M. J. (1989). Phenotypic plasticity and the origins of diversity. Annual Review of Ecology and Systematics, 20, 249-278.

White, M. & Kolb, J. (1974). A preliminary study of *Thamnophis* near Sagehen Creek, California. Copeia, 1974, 126-136.

Yeager, C. P., Burghardt, G. M., & Lyman-Henley, L. (1996). Prey handling in three species of garter snakes (*Thamnophis*): Novelty, interference, and facilitation effects. Manuscript in preparation.

Yoerg, S. I. (1994). Development of foraging behaviour in the Eurasian dipper, *Cinclus cinclus*, from fledging until dispersal. Animal Behaviour, 47, 577-588.

APPENDICES

APPENDIX I

Litters used for each experiment

Chapter	2	2	3	4	4
Experiment	Post-partum	Growth	-	I	II
Litter I.D					
98MF408	X	X	X	X	-
" "546	X	X	X	X	-
" "565	X	-	-	-	-
" "578	X	X	X	X	-
" "579	X	X	X	X	-
" "581	X	X	X	-	-
" "582	X	X	X	X	-
" "583	X	X	X	X	-
" "584	X	X	X	X	-
" "585	X	X	X	X	-
" "586	X	X	-	-	-
" "587	-	-	-	X	-
99MF666	X	-	-	-	X
" "667	X	-	-	-	-
" "668	X	-	-	-	-
" "669	X	-	-	-	-
" "689	X	-	-	-	X
98MM1011	X	X	X	X	-
" "1582	X	X	X	X	-
" "1589	X	X	X	X	-
" "1596	X	X	X	X	-
" "1621	X	X	X	X	-
" "1629	X	-	-	-	-
" "1633	X	X	X	X	-
" "1641	X	X	-	-	-
" "1645	X	-	-	-	-
" "1646	X	X	X	X	-
" "1651	X	X	-	-	-
99MM1666	X	-	-	-	X
" "1723	X	-	-	-	X
" "1727	X	-	-	-	-
" "1729	X	-	-	-	X
" "1731	X	-	-	-	-
" "1753	X	-	-	-	X

Note: X = litter used, "-" = litter not used. 98 and 99 correspond to the year litters were born. MF = McCafferty farm, MM = Miller's marsh. Litter numbers indicate codes given at the Reptile Ethology Laboratory, University of Tennessee.

APPENDIX II

Descriptive statistics by litter, sex, and site for morphological measurements taken on laboratory born neonates in 1998 and 1999.

Litter	Sex	n	Mass	SVL	Tail	HL	JL	HW	IOD
	M	5	2.26(.07)	163.6(1.57)	53.8(.58)	10.24(.09)	10.01(.09)	4.61(.04)	4.02(.04)
98MF408	F	9	2.05(.11)	158.5(2.66)	45.5(1.26)	10.07(.09)	9.98(.12)	4.6(.01)	4.06(.05)
	Total	14	2.17(.07)	161.3(1.63)	50.1(1.58)	10.16(.07)	10.00(.07)	4.61(.02)	4.04(.03)
	M	2	1.64(.02)	150.5(2.5)	45.0(1.0)	9.20(.21)	9.40(.02)	4.39(.18)	3.80(.01)
98MF546	F	2	1.49(.52)	137.0(.15)	42.0(3.0)	9.25(.75)	9.52(1.18)	4.37(.26)	3.81(.19)
	Total	4	1.56(.22)	143.8(2.33)	43.5(1.55)	9.22(.32)	9.46(.48)	4.37(.13)	3.80(.08)
	M	2	1.94(.26)	167.0(0.0)	50.0(2.0)	-	-	-	-
98MF565	F	6	2.16(.08)	165.7(2.14)	48.5(1.02)	-	-	-	-
	Total	8	2.10(.08)	166.0(1.58)	48.9(.88)	-	-	-	-
	M	6	1.77(.03)	151.5(3.47)	50.5(1.26)	9.67(.08)	9.61(.17)	4.47(.06)	3.92(.04)
98MF578	F	2	1.82(.07)	159.0(6.0)	44.5(.50)	10.25(.36)	10.26(.11)	4.64(.14)	4.04(.05)
	Total	8	1.78(.03)	153.4(3.04)	49.0(1.35)	9.81(.13)	9.77(.17)	4.51(.06)	3.95(.04)
	M	2	2.19(.10)	161.0(0.0)	50.0(0.0)	9.88(.25)	9.97(.01)	4.56(.02)	4.14(.12)
98MF579	F	4	2.34(.06)	162.5(2.1)	48.0(.41)	10.06(.09)	10.37(.08)	4.70(.06)	4.13(.05)
	Total	6	2.29(.05)	162.0(1.37)	48.7(.49)	10.00(.09)	10.24(.10)	4.65(.05)	4.13(.04)
	M	1	2.18(0.0)	182.0(0.0)	51.0(0.0)	9.26(0.0)	9.80(0.0)	4.07(0.0)	3.83(0.0)
98MF581	F	1	2.17(0.0)	179.0(0.0)	50.0(0.0)	9.77(0.0)	11.28(0.0)	4.71(0.0)	4.15(0.0)
	Total	2	2.18(.01)	180.5(1.5)	50.5(.50)	9.52(.26)	10.54(.74)	4.39(.32)	3.99(.16)
	M	6	2.04(.07)	166.8(1.47)	50.7(.88)	10.04(.10)	9.97(.14)	4.64(.04)	4.09(.04)
98MF582	F	1	2.19(0.0)	170.0(0.0)	49.0(0.0)	10.19(0.0)	10.57(0.0)	4.84(0.0)	4.14(0.0)
	Total	7	2.06(.06)	167.3(1.32)	50.4(.78)	10.06(.08)	10.06(.14)	4.67(.05)	4.09(.04)

Appendix II
(continued)

Litter	Sex	n	Mass	SVL	Tail	HL	JL	HW	IOD
98MF583	M	2	2.05(.07)	164.0(0.0)	49.0(1.0)	9.76(0.0)*	9.72(0.0)*	4.53(0.0)*	4.17(0.0)*
	F	2	2.01(.07)	162.5(.50)	46.5(1.5)	9.78(.35)	10.03(.08)	4.71(.06)	4.19(.08)
	Total	4	2.03(.04)	163.3(.48)	47.8(1.03)	9.77(.20)	9.93(.11)	4.65(.07)	4.19(.05)
98MF584	M	2	2.42(0.0)	175.0(3.0)	54.5(1.5)	10.13(.32)	9.82(.15)	4.61(.18)	3.87(.04)
	F	1	2.37(0.0)	170.0(0.0)	48.0(0.0)	10.3(0.0)	10.41(0.0)	4.78(0.0)	3.95(0.0)
	Total	3	2.40(.02)	173.3(2.40)	52.3(2.33)	10.19(.19)	10.02(.21)	4.66(.12)	3.40(.04)
98MF585	M	4	1.72(.05)	158.5(3.2)	48.8(1.19)	9.46(.06)	9.64(.09)	4.54(.03)	3.98(.05)
	F	2	1.70(1.5)	151.5(2.5)	45.0(1.0)	9.90(.01)	9.90(.27)	4.54(.12)	4.04(0.0)
	Total	6	1.71(.03)	156.2(2.59)	47.5(1.09)	9.61(.10)	9.72(.10)	4.54(.04)	4.00(.04)
98MF586	M	5	2.18(.06)	165.0(1.18)	46.4(.87)	9.92(.05)	9.81(.09)	4.58(.04)	4.09(.04)
	F	5	2.32(.10)	161.6(2.77)	44.2(1.16)	9.88(.04)	9.67(.07)	4.61(.05)	4.12(.03)
	Total	10	2.25(.06)	163.3(1.53)	45.3(.78)	9.90(.03)	9.74(.06)	4.60(.03)	4.10(.03)
99MF666	M	2	2.39(.03)	175.0(1.0)	53.0(4.0)	9.85(.09)	-	-	-
	F	7	2.44(.07)	170.9(1.28)	46.7(.97)	10.26(.05)	-	-	-
	Total	9	2.43(.05)	171.8(1.16)	48.1(1.36)	10.17(.07)	-	-	-
99MF667	M	3	2.66(.03)	159.3(.88)	48.7(.88)	10.41(.19)	-	-	-
	F	3	2.48(.05)	166.3(2.0)	48.3(.88)	10.52(.10)	-	-	-
	Total	6	2.57(.05)	162.8(1.85)	48.5(.56)	10.47(.10)	-	-	-
99MF668	M	3	2.16(.10)	156.0(2.31)	40.67(1.76)	10.31(.14)	-	-	-
	F	5	2.12(.14)	151.2(2.2)	35.0(1.1)	10.04(.19)	-	-	-
	Total	8	2.13(.09)	153.0(1.75)	37.1(1.36)	10.14(.13)	-	-	-
99MF669	M	4	2.44(.03)	170.3(.75)	49.0(1.78)	10.54(.11)	-	-	-
	F	4	2.33(.07)	169.0(1.47)	43.8(1.7)	10.82(.11)	-	-	-
	Total	8	2.39(.04)	169.6(.80)	46.4(1.51)	10.68(.09)	-	-	-

Appendix II
(continued)

Litter	Sex	n	Mass	SVL	Tail	HL	JL	HW	IOD
99MF689	M	9	1.82(.02)	159.0(1.24)	46.3(1.01)	10.07(.08)	-	-	-
	F	4	1.76(.11)	157.5(.87)	43.8(1.55)	10.16(.17)	-	-	-
	Total	13	1.81(.03)	158.5(.90)	45.5(.88)	10.10(.07)	-	-	-
98MM1011	M	5	2.22(.03)	178.4(2.42)	51.8(.73)	9.90(.08)	9.82(.12)	4.65(.04)	4.01(.04)
	F	3	2.33(.02)	178.7(.88)	49.0(.58)	9.97(.07)	10.09(.08)	4.67(.04)	4.01(.04)
	Total	8	2.27(.03)	178.5(1.48)	50.8(.70)	9.93(.06)	9.92(.09)	4.66(.03)	4.01(.03)
98MM1582	M	4	2.45(.08)	173.3(1.7)	55.3(.48)	10.06(.21)	10.21(.19)	4.76(.05)	4.12(.05)
	F	7	2.43(.03)	169.3(1.23)	51.0(.98)	10.33(.08)	10.32(.08)	4.80(.05)	4.22(.04)
	Total	11	2.44(.03)	170.7(1.12)	52.5(.90)	10.23(.10)	10.28(.08)	4.79(.03)	4.18(.03)
98MM1589	M	1	2.14(0.0)	166.0(0.0)	50.0(0.0)	9.94(0.0)	10.07(0.0)	4.55(0.0)	4.08(0.0)
	F	4	2.23(.18)	164.3(1.80)	47.8(1.11)	10.11(.16)	10.14(.21)	4.65(.07)	4.05(.04)
	Total	5	2.21(.14)	164.6(1.44)	48.2(.10)	10.08(.13)	10.12(.17)	4.63(.06)	4.06(.03)
98MM1596	M	5	1.85(.15)	150.0(5.56)	43.8(1.71)	9.64(.25)	9.58(.21)	4.47(.12)	3.98(.08)
	F	7	1.81(.13)	146.4(3.98)	39.1(2.05)	9.67(.10)	9.89(.17)	4.56(.05)	4.0(.08)
	Total	12	1.83(.10)	147.9(3.16)	41.1(1.50)	9.66(.11)	9.76(.13)	4.52(.05)	4.00(.05)
98MM1621	M	5	2.24(.05)	169.4(1.21)	50.8(1.50)	9.96(.09)	10.05(.14)	4.65(.03)	4.12(.03)
	F	8	2.28(.04)	165.5(1.94)	50.8(.45)	10.28(.07)	10.34(.07)	4.73(.02)	4.20(.03)
	Total	13	2.26(.03)	167.0(1.35)	50.8(.60)	10.16(.07)	10.23(.08)	4.70(.02)	4.17(.03)
98MM1629	M	7	1.98(.04)	163.9(1.60)	49.9(.55)	-	-	-	-
	F	5	1.89(.05)	160.4(1.36)	47.6(.68)	-	-	-	-
	Total	12	1.94(.03)	162.4(1.16)	48.9(.53)	-	-	-	-
98MM1633	M	10	1.90(.05)	163.2(1.63)	49.8(.63)	9.94(.08)	9.96(.10)	4.52(.02)	4.0(.04)
	F	5	1.80(.08)	157.0(1.41)	45.6(.60)	10.20(.09)	10.05(.14)	4.60(.01)	4.08(.07)
	Total	15	1.86(.04)	161.1(1.39)	48.4(.70)	10.03(.07)	9.99(.08)	4.55(.02)	4.03(.03)

Appendix II
(continued)

Litter	Sex	n	Mass	SVL	Tail	HL	JL	HW	IOD
	M	4	2.26(.12)	162.0(1.91)	49.3(1.11)	10.0(.05)	9.91(.08)	4.74(.02)	4.21(.06)
98MM1641	F	7	2.23(.12)	160.0(2.0)	45.4(.84)	9.98(.15)	10.12(.07)	4.80(.02)	4.22(.04)
	Total	11	2.24(.08)	160.7(1.42)	46.8(.86)	9.99(.09)	10.04(.06)	4.78(.02)	4.22(.03)
	M	5	2.41(.05)	162.2(1.85)	48.6(.51)	-	-	-	-
98MM1645	F	4	2.46(.03)	163.3(2.50)	50.3(1.11)	-	-	-	-
	Total	9	2.43(.03)	162.7(1.42)	49.3(.60)	-	-	-	-
	M	3	2.38(.05)	174.0(1.0)	52.3(.33)	10.21(.17)	10.29(.15)	4.73(.10)	4.08(.04)
98MM1646	F	6	2.31(.13)	165.3(1.86)	47.3(.80)	10.07(.10)	10.15(.09)	4.69(.04)	4.03(.05)
	Total	9	2.33(.08)	168.2(1.90)	49.0(.99)	10.11(.08)	10.20(.07)	4.70(.04)	4.04(.03)
	M	6	2.71(.04)	182.0(2.02)	52.7(1.02)	10.0(.06)	9.86(.14)	4.57(.02)	3.92(.04)
98MM1651	F	7	2.64(.04)	172.3(1.66)	47.9(.59)	9.97(.08)	10.02(.07)	4.61(.05)	3.93(.02)
	Total	13	2.67(.03)	176.8(1.86)	50.1(.88)	9.98(.05)	9.94(.07)	4.59(.03)	3.92(1.96)
	M	9	1.50(.04)	150.2(.01)	41.8(.52)	9.95(.08)	-	-	-
99MM1666	F	8	1.58(.02)	148.4(.63)	39.8(.53)	10.07(.09)	-	-	-
	Total	17	1.54(.03)	149.4(.72)	40.8(.44)	10.01(.06)	-	-	-
	M	6	2.21(.05)	172.3(1.28)	46.0(1.03)	9.87(.05)	-	-	-
99MM1723	F	3	2.22(.08)	175.7(4.70)	47.0(1.0)	10.16(.16)	-	-	-
	Total	9	2.21(.04)	173.4(1.68)	46.3(.75)	9.96(.08)	-	-	-
	M	6	3.03(.09)	172.3(2.26)	46.0(.52)	10.51(.12)	-	-	-
99MM1727	F	2	2.09(.48)	151.5(4.5)	33.5(.50)	10.65(.09)	-	-	-
	Total	8	2.79(.19)	167.1(3.88)	42.9(2.08)	10.54(.09)	-	-	-
	M	3	2.69(.14)	173.0(1.0)	48.7(3.84)	10.39(.07)	-	-	-
99MM1729	F	7	2.54(.10)	174.3(1.89)	45.5(.56)	10.80(.16)	-	-	-
	Total	10	2.58(.08)	173.9(1.27)	46.6(1.28)	10.68(.13)	-	-	-

Appendix II
(continued)

Litter	Sex	n	Mass	SVL	Tail	HL	JL	HW	IOD
	M	4	2.68(.12)	162.8(2.84)	43.8(.95)	10.44(.09)	-	-	-
99MM1731	F	6	2.95(.09)	161.7(2.86)	37.3(1.09)	10.64(.12)	-	-	-
	Total	10	2.84(.08)	162.1(1.96)	39.9(1.27)	10.56(.08)	-	-	-
	M	1	2.21(0.0)	159.0(0.0)	45.0(0.0)	10.79(0.0)	-	-	-
99MM1753	F	2	2.33(.07)	164.5(6.5)	45.0(3.0)	10.53(.03)	-	-	-
	Total	3	2.29(.05)	162.7(4.18)	45.0(1.73)	10.62(.09)	-	-	-
98/99MF Totals	**M**		**2.07(.04)**	**162.3(1.04)**	**48.9(.51)**	**9.98(.05)**	**9.80(.05)**	**4.54(.03)**	**4.00(.02)**
	F		**2.16(.04)**	**161.9(1.30)**	**45.02(.60)**	**10.13(.06)**	**10.06(.11)**	**4.63(.03)**	**4.07(.03)**
	Total		**2.11(.03)**	**162.1(.82)**	**47.1(.43)**	**10.05(.04)**	**9.90(.06)**	**4.58(.02)**	**4.03(.02)**
98/99MM Totals	**M**		**2.22(.05)**	**166.1(1.14)**	**48.3(.47)**	**10.04(.04)**	**9.94(.05)**	**4.61(.02)**	**4.04(.02)**
	F		**2.23(.04)**	**162.7(1.08)**	**45.5(.54)**	**10.20(.04)**	**10.13(.04)**	**4.69(.02)**	**4.09(.02)**
	Total		**2.23(.03)**	**164.3(.79)**	**46.8(.37)**	**10.13(.03)**	**10.05(.03)**	**4.65(.01)**	**4.07(.01)**
Grand Totals	**M**		**2.16(.03)**	**164.5(.81)**	**48.6(.35)**	**10.02(.03)**	**9.88(.04)**	**4.58(.02)**	**4.02(.02)**
	F		**2.21(.03)**	**162.4(.83)**	**45.3(.40)**	**10.17(.04)**	**10.11(.04)**	**4.67(.02)**	**4.09(.02)**
	Total		**2.18(.02)**	**163.5(.58)**	**46.9(.28)**	**10.10(.02)**	**9.99(.03)**	**4.62(.01)**	**4.06(.01)**

APPENDIX III

Descriptive statistics (M ± SE) by sex for head length (HL), jaw length (JL), head width (HW), and inter-ocular distance (IOD) at birth, 160 days, and 240 days.

Age	Sex	n	HL(mm)	JL(mm)	HW(mm)	IOD(mm)
Birth	Males	70	9.90(0.04)	9.90(0.04)	4.58(0.02)	4.02(0.02)
	Females	75	10.0(0.04)	10.1(0.05)	4.67(0.02)	4.09(0.02)
160 days	Males	37	10.20(0.06)	10.40(0.06)	5.02(0.03)	4.46(0.03)
	Females	39	10.60(0.07)	10.7(0.05)	5.15(0.03)	4.64(0.03)
240 days	Males	27	10.80(0.07)	11.10(0.06)	5.34(0.04)	4.77(0.04)
	Females	29	11.1(0.08)	11.5(0.08)	5.41(0.05)	4.85(0.04)

APPENDIX IV

Mean (+1 SE) latencies for each consumption phase at FT1, FT2, and FT3 for snakes completing each test in each diet group.

		Diet							
		F (n=10)		W (n=15)		FW-F (n=9)		FW-W (n=11)	
Phase	Test	M(SE)	%diff	M(SE)	%diff	M(SE)	%diff	M(SE)	%diff
Approach	FT1	822.2(313.62)		534.7(125.89)		530.8(230.97)		1292.5(338.03)	
	FT2	336.9(115.70)	59.0	531.8(181.58)	0.5	136.7(47.52)	72.4	883.2(365.28)	31.7
	FT3	301.7(99.12)	63.3	484.2(145.65)	9.4	309.9(150.59)	41.6	282.0(96.82)	78.2
Capture	FT1	1265.8(414.79)		671.3(206.63)		737.9(250.53)		601.0(288.73)	
	FT2	594.9(278.84)	53.0*	386.1(147.79)	42.5*	73.0(48.80)	90.1*	218.8(135.55)	63.6
	FT3	271.3(92.82)	78.6**	136.7(52.20)	79.6**	42.3(8.14)	94.3*	277.3(153.97)	53.9
Handle	FT1	44.0(27.61)		31.2(6.28)		34.6(12.07)		40.6(15.66)	
	FT2	36.6(13.51)	16.8	17.7(4.59)	43.3	75.4(17.59)	-117.9	26.6(6.07)	34.5
	FT3	71.4(34.11)	-62.3	27.8(8.31)	10.1	105.7(27.71)	-205.5	22.9(13.06)	43.6
Swallow	FT1	61.9 (9.71)		90.9(12.95)		66.8(9.52)		57.2(7.88)	
	FT2	75.5(16.03)	-22.0	52.1(8.01)	42.7	63.8(14.76)	4.5	42.5(6.91)	25.7
	FT3	97.8(20.68)	-58.0	63.2(9.49)	30.5	89.9(8.71)	-34.6	52.3(12.49)	8.6
Total	FT1	1371.7(405.34)		793.4(209.54)		839.2(245.46)		698.8(289.01	
	FT2	761.0(285.28)	44.5	455.9(152.82)	42.5	212.2(48.54)	74.7*	287.9(134.59)	58.8
	FT3	440.5(118.21)	67.9*	227.7(56.72)	71.3*	237.9(38.75)	71.7*	352.4(154.12)	49.6

Note: F = Fish group, W = Worm group, FW-F = Mixed group (fish), FW-W = Mixed group (worms). % diff. = percentage decrease between FT1 and FT2, and FT1 and FT3. * = $p < .05$, ** = $p < .001$ significant decrease between FT1 compared with FT2 and FT3

VITA

Mark Andrew Krause was born June 19, 1971, in Walnut Creek California. His parents, Jim and Ruthanne Krause currently reside in Reedley, California where they farm and his brother, Matt Krause, lives in Seattle. Mark shared his room with reptiles throughout most of his childhood, and decided to return to this original love while at U.T., following his five years studying chimpanzee cognition. He received his B. S. and M. S. degrees in Psychology from Central Washington University, before coming to U.T. to work with Dr. Gordon Burghardt in the Reptile Ethology Laboratory. Mark is currently teaching and doing postdoctoral work at the University of Texas at Austin.