To the Graduate Council:

I am submitting herewith a dissertation written by Lani P. Lyman-Henley entitled "Effects of Social and Dietary Experience on Chemosensory Response, Aggregation Behavior and Kin Recognition in the Eastern Garter Snake, Thamnophis sirtalis 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 Life Sciences.

Gordon M. Burghardt

Gordon M. Burghardt, Major Professor

We have read this dissertation
and recommend its acceptance:

J. C. Malone

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

EFFECTS OF SOCIAL AND DIETARY EXPERIENCE ON CHEMOSENSORY RESPONSE, AGGREGATION BEHAVIOR AND KIN RECOGNITION IN THE EASTERN GARTER SNAKE, *THAMNOPHIS SIRTALIS SIRTALIS*

A Dissertation

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Lani P. Lyman-Henley

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DEDICATION

To Damien.

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ABSTRACT

Due to the longstanding and widespread beliefs regarding their lack of sociality, little is known about the aggregative behavior of snakes. A consideration of previous work not only indicates that snake aggregation is a social phenomenon but also suggests a variety of factors that may influence this behavior. A consideration of these factors (diet, familiarity, kinship) form the basis of this research.

Fifty-nine T. s. sirtalis neonates from 2 litters of a Michigan population were measured for mass and snout-vent length, then tested prior to feeding for initial chemical prey preferences and site choice based on conspecific feces. Animals were then placed into group housing situations of 8 balanced for litter, sex, and initial chemical prey preferences, and fed either worms, Lumbricus terrestris (2 groups), or mosquito fish, Gambusia affinis (2 groups). Two groups consisted half of animals on each diet, and 2 additional groups consisted half of T. sirtalis on one diet and half of another species, T. butleri, on the same diet. The remaining animals were maintained in isolation. After a maintenance period, snakes were again measured, tested for chemical prey preferences, and tested for site choice based on both conspecific feces and prey surface cues. The remaining 38

animals not housed with T. butleri were then observed for aggregation behavior in a large arena with 8 identical shelters, after which all animals were again measured.

The main differences in snout-vent length and mass occurred between animals in the different litters, with litter 16 animals being consistently larger than litter 10 snakes. The lack of difference in growth between animals on different diets is assumed to indicate a negligible difference between the health of the subjects.

The T. sirtalis in this research prior to feeding experience showed responses to both fish and worm extract (that were significantly above responses toward a water control), with a preference for worm cues indicated by a greater frequency of predatory attack. Litters showed different initial preferences, litter 10 having a strong worm preference, whereas litter 16 showed no preferece. After experience with prey, shifts of preference were more frequently in favor of the familiar prey, although the strongest effect was again a litter difference. Litter 10 snakes showed no change from their initial preferences, still showing strong worm preferences only slightly decreased by a fish diet, whereas litter 16 animals fed fish acquired a moderate fish preference, while those feeding on worms still showed no preference.

In an Initial Feces-Based Site Choice test, T. sirtalis were shown to have an overall bias in favor of conspecific feces carrying worm cues. In a Final Feces-Based Site Choice test, there was no significant preference for feces-marked sites, though in general animals chose the feces bearing familiar prey cues. There was a difference between the sexes such that males preferred worm cues whereas females preferred fish cues. Sites marked with worm prey surface chemicals were preferred by animals fed worms, snakes in litter 10, and males.

Thamnophis sirtalis formed small, scattered aggregations under several identical shelters. Litter 16 snakes significantly associated with siblings, whereas litter 10 animals were less aggregatory and more active in the arena. Animals from 3 of 6 housing groups associated with familiar animals at a significant rate. There was some evidence that females were stronger aggregators, preferring the same sex, whereas males preferred aggregating with the opposite sex. There was little influence of diet in aggregation behavior, animals on both diets possibly being nearer animals fed worm.

Comparisons were made between the results of different experiments within this research, suggesting that smaller animals preferred worm cues. There was also evidence that

subjects preferred to be nearer animals fed the same prey that was their preference in Prey Surface-Chemical Site Choice. Results support the possibility of information extraction in this species, suggest kin recognition, and possibly individual recognition. Results of experiments with T. sirtalis are also compared with prior work using T. butleri, and further research is suggested to continue our understanding of aggregation in snakes and other animals.

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CHAPTER 1

INTRODUCTION

Aggregation and Social Behavior

Animal aggregations range from those in highly social species, such as ants, to those that arguably have no social interactions at all. Despite its common occurrence among animals, confusion exists in the literature regarding the term "aggregation." Allee (1938), a pioneer in the study of animal aggregation, described aggregations in some species (such as snakes) as merely a simple crowding of nonsocial animals. Scott (1956) suggests that such aggregations are dependent on environmental conditions, and are therefore not truly social. This view of aggregation still remains popular; Immelmann and Beer (1989) define "aggregation" as a congregation of animals that does not depend on social attraction but on habitat selection of favored conditions. This is despite Dundee and Miller's (1968) and Burghardt's (1983) demonstrations that snake aggregation is not due solely to microclimate, since animals aggregated under only a few of otherwise identical shelters in laboratory conditions. In previous work (Lyman, 1990), a use of the term "aggregation" was adopted following Burghardt (1983),

in which the term is used in a purely descriptive sense without allusion to function or causal determinants.

The problem lies not in the definition of aggregation, but in what comprises social behavior, and whether a given aggregation of animals should be considered a manifestation of sociality or not. A variety of definitions of "true" social behavior exist (e.g., Emlen, 1980; Tinbergen, 1953; Wilson, 1975). Among defining factors of social grouping (Wilson, 1975) it has been suggested that two or more animals of the same species must actively come, or remain, together as a group. A second factor to be considered is the minimal length of time that the group remains together. A third criterion that has been suggested is that some minimal amount of time or energy be spent. A fourth concern requires reciprocal communication between individuals. Beyond these basic ideas the occurrence of other factors such as an identifiable social hierarchy, stability of the group across generations, and altruism have also been suggested.

While social behaviors per se (that is, those taken to have interactive significance between individuals) can be found across a wide variety of animals in which there are clearly payoffs and adaptive pressures to be social, a definition such as Wilson's (1975) raises questions about the meaningfulness of aggregations in other species such as snakes, that are generally considered nonsocial but do

congregate (Allee, 1931, 1938; Brattstrom, 1974; Wilson, 1975).

Typically, what distinguishes a meaningful social behavior from a "mere aggregation" has been social functions such as those outlined by Wilson (1975). However, aggregation behavior can confer a suite of advantages to animals regardless of social factors.

Tinbergen (1953) provides several examples of benefits from group living. These include such factors as defense against predators, increased growth, an increased tolerance to adverse environmental conditions, and social facilitation of learning. Tinbergen's discussion takes none of these examples from endothermic vertebrates such as birds and mammals, or eusocial insects such as ants and bees, that studies of social behavior usually emphasize, but rather from goldfish, cockroaches and Daphnia. According to Wilson (1975), additional benefits of group living are increased competitive ability, increased feeding efficiency, reproductive efficiency, survival at birth, and population stability, as well as an ability to penetrate new adaptive zones and modify the environment.

It is clear that most of the above benefits of "social" grouping behavior could also apply to aggregative behavior, whether considered social or not. The differences may not always be readily apparent. If such adaptive outcomes are what should concern us in

determining whether a collection of animals can be said to be meaningfully aggregating or not, then certain species are taken to be asocial that may not exhibit such characteristics as expending energy to remain in social groups, reciprocal communication, identifiable social hierarchies, or altruism. However, it is possible that such animals may enhance their fitness from aggregating without meeting such aforementioned specific criteria.

I suggest that if a given aggregation forms based on the characteristics of other individual animals in the group, and not purely environmental conditions, that it be considered a social response. However, the term "aggregation" should remain descriptive of the behavioral phenomenon of grouping without causal implications. Because snakes have long been described as the least social reptiles (Allee, 1931, 1938; Brattstrom, 1974; Prater, 1933; Wilson 1975), they have proven a good animal in which to consider this phenomenon.

Aggregation in Snakes

Social behaviors seen in snakes include male combat, courtship and mating, including large mating aggregations (Carpenter, 1977; Crews and Garstka, 1982; Finneran, 1949), and communal nesting (Swain and Smith, 1978). Dominance hierarchies have been reported for male Indian

pythons, Python molurus (Boidae) (Barker, Murphy and Smith, 1979), and individual recognition may be found in plains garter snakes, Thamnophis radix (Colubridae) (Yeager and Burghardt, in press). Aggregation of snakes prior to hibernation can be dramatic, wherein hundreds, even thousands, of snakes may converge within a few yards of an annual hibernaculum (e.g., Aleksiuk, 1977; Duvall, King and Gutzwiller, 1985). It can be argued in this case that the attractant is a common resource. But there are also frequent aggregations of snakes outside of hibernation or reproductive seasons that do not show effects of environment (Barboúr, 1962; Dundee and Miller, 1968; Noble and Clausen, 1936). Noble and Clausen (1936) reported that 63% of brown snakes, Storeria dekayi (Colubridae), observed outside of hibernation were in aggregations. A survey of research into snake aggregation and related behavior is provided in Table 1.1.

Several benefits of such aggregation in snakes have been postulated, most of which correspond to benefits of general social grouping. For example, protection from predators has been suggested (Gregory, 1975), reduced oxygen consumption, reduced water loss and increased thermal stability were suggested by Noble and Clausen (1936) in reference to aggregations of Storeria dekayi. Myres and Eells (1968) also report thermal benefits to aggregating Boa constrictor (Boidae), and Aleksiuk (1977)

Table 1.1: Survey of prior research regarding snake aggregations (in chronological order).

Study	Species	Findings
Noble & Clausen (1936)	*Storeria dekayi* *Thamnophis butleri* *T. sauritus* *T. sirtalis*	Thermal, respiratory, humidity benefits of aggregation; vision & olfaction involved
Finneran (1949)	*T. butleri*	Reproductive aggregation
Finneran (1953)	*Agkistrodon contortrix*	Aggregation of gravid females
Fitch (1960)	*A. contortrix*	Aggregation of gravid females
Barbour (1962)	*A. contortrix*	Aggregates feeding on cicada nymphs
Dundee & Miller (1968)	*Diadophis punctatus arnyi*	Habitat conditioning, suggested chemical cues including feces
Myers & Eells (1968)	*Boa constrictor*	Thermal benefits of aggregation
Kropach (1971)	*Pelamis platurus*	Enhanced foraging on fish at sea slicks
Porter & Czaplicki (1974)	*T. r. radix* *Nerodia rhombifera*	*T. r.* prefer conspecific soiled, *N. r.* prefer clean substrates
Gregory (1975)	*T. sirtalis parietalis* *Opheodrys vernalis* *S. occipitomaculata*	Gravid females aggregate, protection from predation
Aleksiuk (1977)	*T. s. parietalis*	Thermal benefits to gravid females
Arnold & Wassersug (1978)	*T. elegans* *T. sirtalis*	Aggregations at anuran metamorphosis sites

Table 1.1: Continued.

Study	Species	Findings
Swain & Smith (1978)	Coluber constrictor	Communal nesting
Barker, Murphy & Smith (1979)	Python molurus	Linear social hierarchy
Scudder, Stewart & Smith (1980)	N. sipedon	Neonates use conspecific chemical cues in aggregations
Crews & Garska (1982)	T. s. parietalis	Review, pheromones in reproduction & hibernation
Golan et al (1982)	Crotalus viridis	Conspecific trailing
Heller & Halpern (1982a)	T. sirtalis	Site fidelity, adult males aggregate
Heller & Halpern (1982b)	T. sirtalis	VNO used in aggregation
Brown & MacLean (1983)	C. horridus	Neonate conspecific trailing
Burghardt (1983)	T. sirtalis S. dekayi	Neonate aggregation, species discrimination
Gillingham, Carpenter & Murphy (1983)	C. atrox	Male dominance & combat
Halpern & Kubie (1983)	T. s. sirtalis T. r. radix	VNO system in feeding, reproduction
King et al (1983)	C. viridis	Avoid conspecific cues
Allen, Burghardt, & York (1984)	N. sipedon N. cyclopion N. rhombifera	N. s. & N. c. prefer conspecific soiled, N. r. clean substrate
Duvall, King & Gutzwiller (1985)	C. viridis	Review, denning agg.

Table 1.1: Continued.

Study	Species	Findings
Graves et al (1986)	C. viridis	Neonates locate dens, conserve water in agg.
Graves & Duvall (1987)	C. viridis	Thermal benefits of agg.
Graves & Halpern (1988)	T. radix	Skin lipid extracts used in aggregation
Schwartz (1989)	T. sirtalis	Heritable agg. tendencies, population differences
Burghardt (1990)	N. sipedon	Enhanced growth in groups
Lyman (1990)	T. butleri	Aggregation effected by diet, familiarity, kinship
Graves, Halpern, & Friesen (1991)	T. proximus	Skin lipid extracts used in aggregation
Yeager & Burghardt (in press)	T. radix	Aggregation with non-competitors, individual recognition

reports similar benefits to Thamnophis s. parietalis. Enhanced foraging has also been suggested in several studies (e.g., Arnold and Wassersug, 1978; Barbour, 1962; Kropach, 1971).

This varied research suggests that the benefits of aggregation within snakes parallel those of accepted social behaviors. Research on a variety of other species has uncovered a number of influences and ideas concerning aggregation that should also be considered. These include the roles of information transfer, kinship and familiarity. Such possibilities have not in the past been thoroughly investigated in snakes, partly due to the widespread assumption that snake aggregations are non-social. Claiming that snakes could be engaging in something like information transfer or extraction concerning diet, kinship or familiarity, requires an understanding of what sensory mechanisms snakes may be using in the reception of such "social" information related to aggregative behavior.

Sensory Control in Snake Aggregation

The role of chemical cues in snake aggregating behavior has been demonstrated by several workers. Dundee and Miller (1968) released ringneck snakes, Diadophis punctatus (Colubridae), in circular, sand-filled laboratory arenas containing ten identical cover objects.

They found that the animals repeatedly showed clumped distributions, and that they preferred to aggregate under shelters previously occupied by other snakes. They called this effect "habitat conditioning" and demonstrated that if the substrate under a favored shelter was translocated, the snakes would not stay under the formerly used shelter, but would eventually start aggregating at the new location of the "conditioned" substrate. An attractant substance was postulated that was integumentary, cloacal, or excretory in origin and was deposited by the first snakes to frequent a site. It was also noted that animals trailed each other between shelters (see also Burghardt, 1980).

Noble and Clausen (1936) pioneered the investigation of the role of the senses in aggregation behavior with the brown snake, Storeria dekayi. They attempted to eliminate vision, olfaction, and/or vomeronasal functioning in their test subjects, influenced by methods used in earlier research of the chemical senses in feeding behavior (Bauman, reviewed in Burghardt, 1970). They concluded that vision, and less extensively olfaction, was the primary sense used in aggregation.

Subsequently, Wilde (1938) provided more carefully controlled work in which the vomeronasal nerves were severed, demonstrating that the VNO system was of primary importance in feeding behavior, and that snakes without VNO function generally did not respond to chemical cues at

all. Later work has supported Wilde's conclusions (Burghardt & Pruitt, 1975; Halpern & Kubie, 1983; Heller and Halpern, 1982b).

Heller and Halpern (1982a) demonstrated that the relevant cues for locating favored sites in eastern garter snakes, T. sirtalis, are left on the substrate by conspecifics. Heller and Halpern (1982b) also demonstrated that T. sirtalis with vomeronasal nerve lesions did not return to previously preferred shelter, supporting the importance of the vomeronasal system in the identification of "conditioned" sites. However, they did note that experimental animals in the presence of normal control animals were able to locate the favored shelters, almost as if using the intact snakes as "guides." A similar finding was reported by Porter, Sentell, and Makin (1987) who found that anosmic spiny mice raised with intact animals did not show the same abnormal behaviors as those raised only with other anosmic pups. Thus, animals can compensate for chemosensory handicaps.

The identity of the exact chemical stimulus for aggregation behavior is still uncertain. Thamnophis radix and T. proximus have been shown to prefer shelters marked with chemical extracts of conspecific shed skins (Graves and Halpern, 1988; Graves, Halpern and Friesen, 1991). Skin has also been cited as the source of conspecific chemical signals in neonate prairie rattlesnakes, Crotalus

viridis (Viperidae) (Graves et al., 1986). Trailing behavior has been noted in other studies of aggregation, and some traces of the integument, probably lipids, seem a likely agent (Dundee and Miller, 1968; Noble and Clausen, 1936; Schell and Weldon, 1985).

Another possible agent to promote aggregation is that of feces, as suggested by Dundee and Miller (1968). Support for this possibility is available in other taxa. Red-backed salamanders, Plethodon cinereus (Plethodontidae), have been shown to use pheromonally marked fecal pellets to identify territories, such that animals preferred burrows marked with their own feces and displayed more at those marked with feces of conspecifics (Horne and Jaeger, 1988; Jaeger, 1986; Jaeger et al, 1986). These salamanders also were able to recognize familiar neighbors, acting less aggressively to them than unfamiliar animals (Jaeger, 1981). They are even able to ascertain size from fecal pellets, and deposit larger pellets near those of same-sized conspecifics (Mathis, 1990).

Several studies have demonstrated that some snakes, including the plains garter snake, T. radix, and water snakes Nerodia sipedon and N. cyclopion (Colubridae) prefer substrate soiled by conspecifics (Allen, Burghardt, and York, 1984; Porter and Czaplicki 1974; Scudder, Stewart, and Smith, 1980). Other snakes such as Nerodia

rhombifera and the prairie rattlesnake, Crotalus viridis, prefer the unsoiled side of the aquarium (Allen, Burghardt, and York, 1984; Porter and Czaplicki, 1974; King et al., 1983). These studies did not control for the occurrence of integumental cues along with feces, and so the role of feces in aggregation has not been adequately investigated.

Regardless of whether it is the excrement itself or other cues, such as integumentary ones, that originally attract snakes to an area occupied by other snakes, it is possible that information transfer may occur via feces. Female salamanders, Plethodon cinereus, have been shown to prefer burrows marked with fecal pellets of males fed termites rather than ants, termites being a preferred food item (Walls, et al., 1989).

Burghardt (1983) used neonate T. sirtalis and Storeria dekayi in aggregation that had not yet eaten and therefore could not have been using feces, let alone dietary cues, to choose sites. However, snakes show responses to chemical cues of prey prior to ingestive experience, but still can be influenced by such experience (as will be discussed below). Thus, that snakes do not need fecal cues to show aggregative behavior does not rule out the possibility that they may use such cues to modify this behavior once available.

Previous work (Lyman, 1990) explored more carefully the possibility that feces, containing information about diet (that is, available prey), could be a basis for information extraction between animals in an aggregation. Such a hypothesis rests on the fact that chemical senses provide important information about prey choice in the first place.

Prey Choice

The vomeronasal system of garter snakes is the single most important sensory modality in finding and identifying prey items (Burghardt, 1967, 1970; Burghardt and Pruitt, 1975; Halpern and Kubie, 1983; Wilde, 1938). Since the snake's flicking tongue functions as a delivery mechanism from the environment to the vomeronasal organ in the roof of the animal's mouth, the rate of tongue-flicking serves as a readily observable measurement of an animal's interest in available chemical cues. It has been shown that tongue-flick rates correspond to a snake's activity level (Chiszar and Carter, 1975; Gove and Burghardt, 1983) and increase in the vicinity of natural prey items (Burghardt and Denny, 1983; Carr and Gregory, 1976; Chiszar, Scudder and Knight, 1976). Burghardt (1966), investigating chemical prey preferences of garter snakes, developed the Tongue-Flick Attack Score (TFAS), that utilizes rate of tongue-flicking combined with the

latency to attack, where high tongue-flick rate and short latencies indicate predatory interest in a stimulus. Cooper and Burghardt (1990) compared the TFAS to several other measures of interest in chemical prey cues and found TFAS to be a highly successful indicator of snake interest in chemical prey cues. Related methodological considerations such as the most effective preparation of surface extracts of prey, and the nature of the substances eliciting prey attack behavior in naive snakes have also been investigated (Schell, et al., 1990; Sheffield, Law and Burghardt, 1968).

Neonate garter snakes respond to aqueous surface extracts of prey species without prior experience. Burghardt (1969) demonstrated that five species of garter snakes show differing levels of responsiveness to varying prey species and that these profiles generally agree with documentation of natural diets. Across three subspecies of eastern garter snake, T. sirtalis, the highest responses were generally given to extracts of earthworms, but much interest was shown to fish, amphibian, and leech extract as well. The plains garter snake, T. radix, showed a similar profile. Both of these species are dietary generalists. Thamnophis butleri was somewhat unusual in that naive snakes responded to worm, leech, fish and amphibian extracts, though these animals are not known to feed on fish, and rarely on amphibians, in the

wild. The northwestern garter snake, T. ordinoides, showed a higher response to slug extract than any other, and the aquatic garter snake, T. couchii, responded most strongly to fish and amphibian extracts. Carr and Gregory (1976) report similar findings with western populations of T. sirtalis, T. elegans, and T. ordinoides.

There can be prey preference polymorphism within litters of T. sirtalis in which individuals show preferences for different prey types (Burghardt, 1975). Arnold (1977, 1981) has demonstrated that inexperienced animals from different populations of the coast garter snake, T. elegans, show differing propensities for surface extract of the slug, Ariolimax californicus, and that these differences have a genetic basis.

There is no effect of maternal diet on the responses of newborn snakes to chemical cues (Burghardt, 1971). However, experience with various prey can effect changes in the initial preferences of garter snakes. Burghardt (1978) states that although the initial recognition of chemical cues for prey seems innate, nothing is thereby implied about the modifiability of such recognition after birth. Arnold (1978) demonstrated that experience with live fish increased later responsiveness to dead fish in neonate T. sirtalis, and that animals fed exclusively on fish showed higher responses to chemical extracts of fish than did animals raised on frogs.

Fuchs and Burghardt (1971) had previously shown that not only could garter snakes discriminate between different types of prey such as fish and earthworm, but that they could develop the ability to discriminate between different species of the same prey type. In this experiment, T. sirtalis that were fed redworms (Eisenia foetida) became able to discriminate between surface extracts of the familiar redworms and unfamiliar nightcrawlers (Lumbricus terrestris) after only eight feedings. These animals showed a greater response to the familiar prey than to the unfamiliar worm species used. Animals that were fed fish paralleled this pattern, with stronger responses to the familiar species of fish.

Investigations of Aggregation in Garter Snakes

The aggregative behavior of snakes, its possible functions, and sensory components have been summarized above. The vomeronasal senses have been demonstrated to play an important role in both aggregation and response to prey cues. From the review of the above studies a program of research was developed to consider such factors as kin and sex along with a focus on diet within an investigation of cues used in aggregative behavior. Chemical cues, possibly transmitted by feces, could be a vehicle for the transfer of information along these dimensions, and could provide new information on aggregation in snakes.

In an experiment using neonate plains garter snakes, Thamnophis radix, G. M. Burghardt and I investigated the correspondence of chemical prey preferences with habitat choice (summary in Burghardt, 1990; Burghardt and Lyman-Henley, in preparation) in an attempt to explore the possibility of information transfer in garter snakes.

The subjects were from one litter, and were raised in four groups of six, two groups of which were fed only earthworms, Lumbricus terrestris, and two fed only mosquito fish, Gambusia affinis (Poeciliidae). The animals were tested for chemical prey preferences when only a few days old, before being fed or assigned to groups. They were retested at 38 weeks of age. They were also tested for site choice based on prey surface cues, where the two prey items were rubbed on substrate under shelters on either side of a test box. Most importantly, they were tested for site choice based on feces from unfamiliar conspecifics feeding on the different prey items. Growth rate was also tracked as an indicator of general health of the animals on each diet.

As expected, chemical preferences favored the familiar diet. Habitat choice based on prey surface cues also favored the familiar prey cues, more noticably in the worm-fed animals. However, both diet groups preferred the side marked with fish-based feces, again most noticably in the worm-fed animals. This preference for fish-based

feces seemed not to be related to growth, since animals fed worms grew more rapidly than those fed fish. A preference for prey other than that recently experienced has been shown in chameleons (Chamaeleo senegalensis) fed on different species of insects (Eason, 1990). However, such responses are often due to a lack of nutritional balance in the diet. Studies using 4 species of mice demonstrated that animals fed only upon differently flavored grains showed the same preference for novel food, a finding similar to the chameleons, but if the flavors were offered concurrently with a nutritionally balanced background diet, animals preferred the familiar flavor (Partridge, 1981; Partridge and Maclean, 1981). In the T. radix study here discussed, the lack of difference in growth rate implies a negligible difference in nutrition between the two diets, and chemical prey preferences were toward the familiar prey. However, this does not exclude the possibility that this phenomenon may still function regarding the contrary choices based on conspecific feces cues, although it does seem unlikely in the absence of a similar response to the chemical prey cues.

The intriguing result regarding conspecific feces cues implies that plains garter snakes are able to discriminate between feces from conspecifics on different diets and use this information to choose habitat sites. However, the attraction seems to be in favor of sites marked by

animals feeding on the non-preferred diet. Such preferences suggested the plausibility of information transfer and invited a line of continued research.

A series of studies were then designed and conducted to explore and develop this possibility in neonate Butler's garter snakes, Thamnophis butleri (Lyman, 1990). A brief overview of the methods and results of that work should provide an appropriate introduction into the proposed plan of research for this dissertation.

The animals used were Butler's garter snakes, Thamnophis butleri, born to two females collected in Wayne County, Michigan. This species was chosen based on its close phylogenetic relationship to T. radix (Conant, 1950; Davis, 1932; Ruthven, 1908; Schmidt, 1938) which were used in the preliminary study (Burghardt, 1990; Burghardt and Lyman-Henley, in preparation), and its tendency to aggregate (Carpenter, 1952a; Finneran, 1949; Noble and Clausen, 1936). This animal is also unusual in that it is a worm specialist in the wild that will feed on fish in captivity (Halloy, 1987; Ruthven, 1908). It was believed that this would provide an additional aspect to the dietary experience portion of these experiments as this species may have a very strong natural bias toward earthworms regardless of experience.

Thirty-eight T. butleri neonates from 2 litters were measured, then tested prior to feeding for initial

chemical prey preference and site choice based on conspecific feces. Snakes were isolated within 24 hours of birth to minimize familiarity with siblings prior to experimental procedures. Animals were placed into group housing situations of 8 snakes balanced for litter, sex, and initial chemical prey preference. Two groups were fed only earthworms (Lumbricus terrestris), two groups fed only mosquito fish (Gambusia affinis), and two groups contained four animals on each diet. Two additional groups consisted of 4 T. butleri housed with 4 T. sirtalis neonates on the same diet (one group on each diet). The remaining six animals were housed individually. After a maintenance period, snakes were again measured, tested for chemical prey preferences, and tested for site choice based on both conspecific feces and prey surface cues. The animals not housed with T. sirtalis were then observed for aggregation behavior in a large arena with 8 identical shelters, after which all animals were again measured.

Data were obtained on growth, and indicated that the main differences in mass and snout-vent length were between the sexes, males being both heavier and longer than females throughout the study. The lack of substantive differences in growth between diets should translate into a negligible difference in the health of the subjects.

The T. butleri in these experiments showed a preference for earthworm extract over fish extract or a water control prior to feeding experience. The final result after experience with prey was that T. butleri that were fed worms responded much more strongly to worm than fish cues, whereas snakes fed fish showed high responses to both prey, slightly favoring fish, and responding significantly more to fish than did worm-fed animals.

In the Initial Feces-Based Choice Test, the animals were shown to have an overall bias in favor of feces carrying worm cues. However, in the Final Feces-Based Choice Test, animals fed fish continued to prefer worm-based feces, while those fed only worms shifted preference toward sites marked with the unfamiliar fish-based feces. There were no preferences demonstrated toward sites marked with prey surface cues.

Thamnophis butleri readily aggregated under preferred shelters. Specific pairs of animals could be identified which were more closely associated than others, and these pairs tended to consist of unfamiliar littermates (those that had been separated since shortly after birth) on opposite diets. Animals with very high or low association rates could also be identified. Correlations between various measures demonstrated that measures of growth interacted with aggregation behavior, such that larger

snakes had smaller distances separating them from their nearest neighbors.

It was suggested from these findings that familiarity (or lack thereof) may be the single most important factor in aggregative behavior, with dietary experience and kinship playing secondary roles. The interactions of dietary experience with the feeding specialization of T. butleri, and possibly ancestral chemical preferences were also discussed.

Kinship, Familiarity and Diet in Aggregation

A major theory in the evolution of social behavior is that of kin selection, whereby animals increase their own inclusive fitness by aiding near relatives (e.g., Hamilton, 1964). There is now a vast literature dealing with the topic of kin selection, and the associated possibility of kin recognition in various animal species. The most frequently inferred mechanisms of kin recognition are phenotypic matching, in which an individual compares unfamiliar animals to a template based on itself or "known" relatives, and familiarity with litter- or nest-mates through repeated contact (Ferkin, 1990; Holmes and Sherman, 1983). In some cases, proximity and familiarity are good predictors of relatedness, and in those cases special mechanisms for recognizing kin are unnecessary

(Ferkin, 1990; Holmes and Sherman, 1983). However, this is not always the case, and much research has been performed in trying to determine whether various animals are capable of discriminating between near relatives, and using what cues. Also, the level of kinship to which such behavior is directed may vary.

Of course, no terminology is without controversy, and kin recognition is surrounded with its fair share of debate. Grafen (1990) argues that an ability to discriminate between conspecifics based on genetic similiarity is incidental to species recognition and does not imply kin recognition. His seems to be a limited use of the term "kin recognition" that requires some purposefulness on the part of the animals, and he states that "systems have not evolved to assess kinship, are not used to assess kinship in nature, and that the ability to assess kinship is too weak to be useful" (Grafen, 1990, p. 53). On the contrary, there seems to be ample evidence that any behavior that confers a selective advantage, be it by kin selection or any other edge, is "useful" in nature regardless of its initial "purpose." I prefer to use the term in the more common, broader, sense that refers to the phenomenon in which animals behave in a manner indicating that they can discriminate between conspecifics on the grounds of relatedness.

Kinship and Sibling Recognition

Waldman and Adler (1979) argue that gregariousness of toad tadpoles may function to enhance feeding efficiency, thermoregulation, predator deterrence, or serve an aposematic function, since these animals are strikingly colored and distasteful to predators. A preference for siblings within these aggregations is consistent with a kin selection model such that advantages are conferred to siblings within a swarm of distasteful tadpoles. Growth rate and speed of metamorphosis of such tadpoles is also density-dependent, such that growth rate increases with density until food becomes a limiting factor (Wilbur, 1977), at which point interference competition may occur (Steinwascher, 1978).

Aggregations of American toad tadpoles, Bufo americanus (Bufonidae), that were marked and released demonstrated that 64% of schools were biased in favor of one sibship group, and suggested that preferences may be formed even prior to hatching (Waldman, 1982). In an early experiment, laboratory-reared tadpoles clearly preferred siblings if reared alone or with siblings, but if reared in mixed groups discrimination was not made unless there was very early exposure only to siblings (Waldman, 1981). In later work, tadpoles did not discriminate between familiar and unfamiliar siblings in preference (Waldman, 1985), and association preferences

were shown to be based on waterborne cues (Waldman, 1986). Waldman (1984) also demonstrated that wood frog tadpoles, Rana sylvatica (Ranidae), reared either with siblings or mixed groups of siblings and nonsiblings preferred to associate with familiar siblings over familiar nonsiblings, but that this preference did not continue after metamorphosis (Waldman, 1989).

Blaustein and O'Hara (1987) showed that wild-caught Cascade frog tadpoles, Rana cascadae, prefer to associate with members of their natural aggregation over members of other aggregations from the same pond. Furthermore, lab-reared subjects preferred to associate with groups containing full siblings than either groups of non-siblings or empty compartments. Tadpoles reared with siblings preferred siblings. Interestingly, tadpoles reared in mixed groups of siblings and nonsiblings preferred to associate with groups of pure siblings over mixed groups of siblings and familiar nonsiblings, and preferred to associate with unfamiliar siblings over unfamiliar nonsiblings (O'Hara and Blaustein, 1981). Tadpoles reared with nonsiblings also show a preference for siblings over nonsiblings, demonstrating that experience with siblings is not a necessary prerequisite for the development of sibling recognition in this species (Blaustein and O'Hara, 1983). Tadpoles reared in isolation also preferred to associate with siblings,

suggesting that the ability to discriminate between siblings and nonsiblings may have some innate component (Blaustein and O'Hara, 1981). The ability to discriminate between siblings and nonsiblings in Rana cascadae tadpoles was shown to be based on waterborne chemical cues sensed by olfaction or taste (Blaustein and O'Hara, 1982a), and persists past metamorphosis at least 47 days (Blaustein, O'Hara, and Olson, 1984). Rana cascadae tadpoles were also shown to utilize cues of maternal or paternal origin in distinguishing siblings from half- or non-siblings, preferring maternal cues (Blaustein and O'Hara, 1982b). These varied data are consistent with both phenotypic matching or genetic recognition system of kin recognition, and suggest both learned and innate components.

Such preferences for siblings regardless of familiarity can be found in animals other than tadpoles, as well. Gregarious Japanese quail chicks, Coturnix c. japonica, have been shown to prefer to associate with unfamiliar siblings over familiar nonsiblings only 4 days after hatching (Waldman and Bateson, 1989). Female house mice, Mus domesticus (Muridae), have even been shown to discriminate between certain males with different genotypes (Coopersmith and Lenington, 1990).

Lab-reared female Belding's ground squirrels, Spermophilus beldingi (Sciuridae), are less aggressive to

full siblings than half-sibs, and less aggressive to half sibs than unrelated squirrels, parallelling social favoritism directed to closely related female conspecifics in the wild (Holmes, 1986a). However, this species showed an ability to also discriminate the kin of unrelated nestmates, showing an effect of familiarity and evidence for learning in phenotypic matching (Holmes, 1986b). In another experiment, particularly relevant to the present research, hatchling green iguanas, Iguana iguana (Iguanidae), were found to prefer unfamiliar siblings over non-sibs based on feces odor, the tightest groups containing familiar siblings (Werner, et al., 1987).

Familiarity and Kinship

Porter, Matochik and Makin (1986) demonstrated a kin preference in spiny mice, Acomys caharinus, that was accentuated by familiarity, while white-footed mice, Peromyscus leucopus (Muridae), and deer mice, P. maniculatus, preferred familiar animals regardless of genetic relationship (Halpin & Hoffman, 1987; Dewsbury, 1988a, 1988b, respectively). Similarly, female meadow voles, Microtus pennsylvanicus, in the breeding season prefer odors of male voles (Ferkin and Seamon, 1987; Ferkin and Zucker, in press), while responses to familiar females are more amicable and less agonistic than those

toward unfamiliar animals regardless of genetic relatedness (Ferkin, 1988; Ferkin and Rutka, 1990).

In the social systems of many animals, including voles and deer mice, familiarity virtually guarantees relatedness, since nestmates are always full siblings (unlike the situation in which several clutches of frog eggs are laid in close proximity within the same pond). Therefore, though association is not a reliable basis for kin recognition, in many systems it may serve the same function (Ferkin, 1990).

Optimal Outbreeding, Age and Sex

When mate choice becomes involved in associative preferences of animals, some of the dynamics differ from associations between neonates. In a series of experiments with Japanese quail, Coturnix c. japonica, a preference was established for first cousins. Birds preferred to mate with members of the opposite sex that were slightly different from individuals that they had been reared with (Bateson, 1982). They preferentially approached unfamiliar animals over those raised with, but this was effected by the number of opposite sex individuals that had been experienced. Too few models and novel birds were likely to be too strange, too many models and the novel bird was likely to be too similar to a familiar animal (Bateson, 1980). This preference for similar, but

unfamiliar mates functioned regardless of actual relatedness (Bateson, 1978). This effect does not require gonadal hormones during period of exposure to produce said patterns of sexual imprinting (Hutchison and Bateson, 1982).

Preferences for mating with males related at the level of first cousin can also be found in zebra finches, Taeniopygia guttata (Aves: Estrildidae), regardless of prior experience (Burley, Minor and Stracham, 1990). Female field crickets, Gryllus bimaculatus, evade or even attack courting male siblings (Simmons, 1989) based on pheromonal cues (Simmons, 1990). Crickets prefer males of intermediate relatedness over unrelated males, in other words, cousins (Simmons, 1991).

Barnard and Fitzsimons (1988, 1989) demonstrated that male mice, Mus musculus, prefer females that are second cousins (even better if familiar), which combination produces relatively large litters of aggressive male offspring. It had earlier been shown that aggressive males were more likely to mate after emigration (Baker, 1981). Keane (1990a, 1990b) also demonstrated that matings with males of intermediate relationship, "first cousins," showed the least inbreeding (or outbreeding) depression, and females refused to mate with closer kin.

Age and sex have also been shown to influence grouping behavior in house mice, Mus musculus, such that juvenile

female mice associate with other females, but as they reach sexual maturity they begin to be attracted by male urinary odors instead (Drickamer, 1989). In fact, while female Mus are attracted to urine odors of the resident male, unmated daughters persistantly invade neighboring males' territories (Hurst, 1990). Mate choice can also be enhanced by familiarity alone, such as the case with Merriam's kangaroo rats, Dipodomys merriami (Heteromyidae), frequenting sandbathing sites (Randall, 1991).

Functions or benefits of aggregative behavior other than those expounded above may also be involved, of course. One of these possibilities is the occurrence of information transfer (or extraction) between members of a group.

Information Transfer

Ward and Zahavi (1973) proposed the "information center" theory in which it was noted that roosting birds somehow shared information regarding the location of distant foraging sites. Altmann and Altmann (1970) had described a related behavior in which yellow baboons touched muzzles as if to smell what was in the other's mouth, suggesting that information could be spread in this manner throughout the troop. Such actions may or may not be considered social behavior depending on whether

reciprocity in communication is required. In either case, it has been very difficult to demonstrate its occurrence in nature (Götmark, 1990).

Galef (1986a, 1988) and associates have subsequently demonstrated this phenomenon of information transfer in Norway rats, Rattus norvegicus (Muridae), which have a central nesting system in the wild. Rats trained in a maze learned spontaneously to follow other rats to food rewards, and were more likely to follow leaders having recently fed on a desirable food (Galef, Mischonger and Malenfant, 1987). Subjects (observers) were allowed to interact with another rat (demonstrator) that had just fed on either cinnamon or cocoa flavored food. When given a choice of the two flavors, observer rats were more likely to choose the same flavor that the demonstrator rat had eaten. This held true even if the demonstrators were anaesthetized or dead at the time of "interaction," and the information was found to be transferred via olfaction (Galef and Wigmore, 1983).

Social factors were found to be more important in this phenomenon than nutritional content of the food (Beck and Galef, 1989) or nutritional needs of the animals, palatability of the food, or required handling time (Galef, 1986b). Animals did not need to be food deprived, and familiarity of the demonstrator was unimportant (Galef, Kennett, and Wigmore, 1984). Recently, Galef

(1991) has demonstrated that if both animals in such an interaction had just eaten a different flavor, they would "exchange" information such that each would choose the flavor the other rat had eaten over the one they themselves had eaten. These effects were shown to be based on social factors and not just exposure to the food cues (Galef, Kennett, and Stein, 1985). There is also some evidence that such food preferences in rats may be affected by maternal diet during gestation (Hepper, 1991).

Such information can apparently also be used to choose associates rather than diet itself. Porter, McFadyen-Ketchum and King (1989) reported that spiny mice, Acomys caharinus, associated more often with other mice that had been fed the same natural or artificial diet.

The Present Study

I have described above research in which I began investigating patterns of aggregation in juvenile garter snakes to discover an apparent interaction of factors including diet, familiarity and kinship. Clearly there is evidence that any one, or combination of, these factors may indeed be occurring and could confer selective advantages upon aggregating snakes. However, the relative importance of these factors and the evolution of the

aggregative behavior of different species of garter snake requires further investigation.

To follow up on this work the following specific hypotheses will be evaluated with the eastern garter snake, Thamnophis sirtalis. Based on the ideas of information transfer and kin recognition, previous ecological studies of this animal, and the results of the prior studies described, the following hypotheses were formulated:

1. Thamnophis sirtalis will respond to both worm and fish surface extracts, as both are part of the species typical diet. This has been previously demonstrated in T. radix, another dietary generalist, and in other populations of T. sirtalis.

2. Thamnophis sirtalis will increase in responsiveness to chemical prey cues after dietary experience, in favor of the familiar prey animals. This has been supported in various species of Thamnophis.

3. Thamnophis sirtalis will choose sites marked with preferred chemical prey surface cues. This would make ecological sense, since prey should be more likely to be found where its chemical cues are strongest. This has been demonstrated with T. radix, although not with T. butleri. I propose that the dietary similarity between T. sirtalis and T. radix will allow a similar finding here.

4. Thamnophis sirtalis will prefer sites marked with feces of same-sex conspecifics fed an unfamiliar diet. Neither T. radix nor T. butleri showed a preference for sites marked with feces of animals feeding on a preferred diet; indeed, they preferred the feces of animals on an unfamiliar diet. This implies a completely different function to the behavior than that initially supposed, which was a direct correspondence with prey preference in order to locate prey.

5. Thamnophis sirtalis will aggregate preferentially with unfamiliar littermates feeding on the unfamiliar diet. There is a lack of information regarding aggregation patterns based on diet, kinship, sex, or familiarity in T. sirtalis. Therefore, assuming that the previous four hypotheses are correct and the underlying mechanisms of the behavior are similiar, I tentatively predict that T. sirtalis will parallel T. butleri in aggregation pattern.

From these data two important types of result can be obtained. First and more generally, does the model of information transfer as presented, researched and discussed in the thesis using Thamnophis butleri continue to provide a framework for describing the behaviors of Thamnophis sirtalis? Second, to the degree that these species (and T. radix) are both similar and different, data will be obtained for a discussion of the comparative

behaviors of these three garter snake species. Given that results obtained in the original work with T. radix and subsequent work with T. butleri paint a somewhat complex picture of the nature of information transfer and aggregation, these proposed studies with T. sirtalis are a logical extension of this research program.

A report of results from the execution of this program of research follows in the remainder of this dissertation. Chapter 2 will discuss general methodology (e.g., origin of animals, housing, feeding). Chapter 3 includes growth information about the subject animals relative to the experimental regime. Chapter 4 details how dietary experience effects chemical prey preference as measured through standard prey surface-extract testing. Chapter 5 examines site choices made by the animals based on cues from prey and conspecifics. Chapter 6 involves arena observations of aggregation behavior in the snakes. Chapter 7 concludes the thesis with a consideration of possible interactions among key variables and a final discussion of the results with respect to how they advance our understanding of aggregrations in snakes with respect to information transfer, kinship, and familiarity.

CHAPTER 2

GENERAL METHODOLOGY

Overview

This chapter briefly describes the natural history of Thamnophis s. sirtalis, and then details the origin, identification, housing and diet of the subject animals used in this study. Particular experimental methods are presented within the chapters detailing individual studies.

Species Description

The eastern garter snake, Thamnophis sirtalis, is found throughout most of the United States and southern Canada, being absent only from more arid areas of the south and western U.S. (Conant and Collins, 1991; Fitch, 1965). Fitch (1965) also notes that in most of its range, T. sirtalis occurs with at least one other species of garter snake, and usually occupies the more general ecological niche.

Wright and Wright report in 1957 that T. s. sirtalis are described as being found "everywhere," including woods, near water (both lakes and rivers or streams), meadows, marshes, swamps, gardens, roadsides, ditches,

parks, and clearings. It may be the case that in the ensuing 34 years the eastern garter snake has become somewhat less ubiquitous. Carpenter (1952a) describes the microhabitat of this species in southeast Michigan as being open hillsides and some wooded areas.

This species of garter snake has been reported to feed on amphibians, earthworms, fish, leeches, small mammals, and birds, with occasional (and controversial) reports even including snails and insects (Carpenter, 1952a; Conant and Collins, 1991; Fitch, 1965; Lagler and Salyer, 1945). Fitch (1965) states that T. sirtalis is found in diverse habitats throughout its range and probably displays some geographic variation in food habits beyond that enforced by availability. When available, T. sirtalis is not above feeding on somewhat unusual prey such as trout (Lagler and Salyer, 1945).

Subjects

Subjects for these studies were derived from 2 litters. Gravid females were collected in Wayne Co., Michigan, by a commercial dealer and arrived at the University of Tennessee at Knoxville on June 2, 1988. Female T. sirtalis were housed at an ambient temperature of 30°C. Neonates were born on June 27, 1988 to 2 females lab coded 88-MI-1610 (N=31) and 88-MI-1616 (N=36). Litters will be referred to from here on only by the last

two digits of this lab code, which reflects maternal identity.

Gravid females had been used in an investigation of the possible effects of incubation temperature upon the sex ratio of offspring, since such has been shown to be the case in several reptile species (Bull, 1985; Burger and Zappalorti, 1988). Gestating female garter snakes demonstrate very precise thermoregulatory behavior, and tend to prefer body temperatures ranging from 25°to 32°C (Huey et al., 1989). It has been shown that different incubation temperatures can have effects on the behavior of neonate snakes (Burger, 1990; Burger, Zappalorti and Gochfeld, 1987). However, I believe that the lack of a temperature gradient would not have had significant detrimental effects on the offspring of these females, considering that the temperature that these test snakes were kept at was within the preferred temperature range for gestating Thamnophis. Sex ratios were not affected by gestation at 30°C (Schwartz, unpubl.), and animals appeared to be healthy with normal behavior and responsiveness to testing procedures for their species and population.

At birth all neonates were removed from the maternal container and temporarily held in a 15 x 30 x 9 cm clear plastic vivarium with damp paper substrate. Animals were removed one at a time, in no specific order, and assigned

a subject letter in alphabetical order from A through Z, then AA to AJ, per litter. Animals were weighed and measured (see also Chapter 3), and placed in individual clear plastic containers (13 x 18 x 5 cm). Each container contained substrate and folded "tent" shelter of Aniboard© (a green paper board treated with neomycin to control bacteria and mold) and a glass water dish. All snakes in containers were visually isolated from one another by paper dividers. Animals were kept at 20±2°C on a 12:12, light:dark, photic cycle. The neonatal T. sirtalis were sexed by hemipenal eversion at birth by J. M. Schwartz, and were rechecked at 25 days of age by G. M. Burghardt.

Dietary Regimes

Neonates were placed into one of two dietary regimes; either fish (Gambusia affinis) or earthworm (Lumbricus terrestris). Food was first offered to neonates at 14 days of age. Fish were presented first to approximately half of the snakes, balanced for litter, sex, size, and Initial Chemical Prey Preference (see Chapter 4), with the rest offered worms first. If the initial prey was not accepted by the third offering, made every other day, the opposite prey was offered. Once a prey item was accepted, snakes were from then on fed only that prey.

Only three animals refused the first offered prey. In order to maintain balanced dietary groups, one other animal was switched between groups after having eaten. This resulted in a total of four animals that were exposed to both prey before final testing, only one of which had actually eaten both. See Table 2.1 for composition of diet groups, and individuals exposed to opposite prey.

Prey animals

Mosquito fish, Gambusia affinis, were collected from wild populations using a seine and hand nets. Fish were maintained in the laboratory in aquaria and fed commercial flake food until use (collection and maintenance as in Lyman, 1990).

Nightcrawler earthworms, Lumbricus terrestris, were purchased from a local commercial dealer and kept in their original soil within a refrigerator at approximately 5°C until use. Worms were occasionally fed corn meal and vegetable matter (e.g., carrot, apple, zucchini shavings).

Feeding Protocol

Fish were offered while still alive in water dishes. Worms were offered as freshly cut pieces on a 55 mm plastic petri dish. Lumbricus had to be cut due to the large size of the intact worm. Recalcitrant eaters were sometimes offered prey on forceps as well, especially

Table 2.1: Composition of groups of *Thamnophis s. sirtalis* (N=59) detailing early exposure to prey items.

			SUBJECT CODE	
GROUP	DIET	LITTER	MALES	FEMALES
1	FISH	10	D, K	N, AB
		16	Y, AH*	V, AF
2	FISH	10	S, V	Q**, AD
		16	J, AE	S, AD
3	WORM	10	I, X	Z+, R
		16	X, AC	E, K
4	WORM	10	A, F	C*, AA*
		16	G, H	C, I
5	FISH	10	L	M
		16	AB	R
	WORM	10	Y	P+
		16	U	AJ
6	FISH	10	W	T
		16	M	T
	WORM	10	AE	B
		16	W	AI
(mixed species with *T. butleri*)				
10	FISH	10	-	H, J
		16	B, Z	-
11	WORM	10	-	O, AC
		16	F, L	-
(individually housed animals)				
"12"	FISH	16	-	AA, P
	WORM	16	-	D

NOTE: "*" denotes animals exposed to both prey items (N=3); "**" denotes animals that had eaten both prey items (N=1); "+" denotes animals added to groups within a month of initial testing. Letters are subject I.D. codes.

early in the experiments, to insure acceptance. All animals, once accepting food readily, were fed twice weekly in their individual containers.

Gambusia offered to fish-eating snakes ranged in length from 6 mm to a maximum of 25 mm total length, with an estimated mean of 13 mm. One to five, modally two, fish were offered per feeding, averaging 0.15-0.20 grams per meal. Pieces of Lumbricus offered to worm-eating snakes ranged from 5 mm to 10 mm in length and 2 mm to 4 mm in diameter. One to four pieces were offered, averaging 0.20-0.30 grams per meal. Mass was measured for different length prey items, and meal sizes were then approximated. Since size and number of available Gambusia varied greatly depending on collecting success, worm meals were measured out to be proportional to the available fish meal.

All snakes were given a vitamin-mineral supplement in their water twice monthly. This was done by using the commercial bird preparations Avitron© and Avimin© . Worm pieces for the animals on the worm-only diet were dusted with Lambert Kay© calcium phosphorus with bone meal every third feeding, since previous research (detailed in Lyman, 1990) suggested worms were much lower in content of these minerals than fish.

Group Housing

Once neonates were regularly accepting food, they were assigned to one of eight housing groups, each group containing eight snakes, equated for mass and balanced for sex, litter, and initial chemical prey preference (described in Chapter 4). Groups were housed communally (except for feeding periods) in 15 x 30 x 9 cm clear plastic boxes with Aniboard© substrate, a plastic shelter, and a glass water dish.

Two groups were made up of T. sirtalis on fish diets (groups 1 and 2), another two contained animals on worm diets (groups 3 and 4), and two more consisted of four animals from each diet regime (groups 5 and 6). The last two groups contained four snakes each of two species. One of these groups was on a fish diet (group 10), the other on a worm diet (group 11). The second species present were Butler's garter snake, Thamnophis butleri, originating from two litters collected from the same area in Michigan as the T. sirtalis, that were six days younger. Thamnophis butleri subjects were those utilized in the aforementioned thesis and comprised groups 7, 8, and 9 (Lyman, 1990). The two-species groups were utilized in a comparative study that will not be detailed here.

Five of the remaining animals were maintained in isolation (group 12), one was removed from the experiment

due to a slight throat deformity, and the remaining 7 had died before group housing was begun. Two animals were substituted in from group 12 to replace animals that died in groups 3 and 5 within a month of initial testing, lowering the number of animals in group 12 to 3. Housing group compositions are detailed in Table 2.1 (p. 42).

Identification of Subjects

All animals were permanently marked for identification by clipping of the belly scales (ventral scutes). Such clipping is frequently used in mark-recapture studies and rarely if ever provides a site for infection (Brown and Parker, 1976). The procedure seems to cause minimal discomfort, since snakes will accept food without prior fasting immediately after marking (Lyman, personal observation).

Thamnophis sirtalis were marked at 22 to 24 days of age. Snakes were held firmly under a dissecting microscope and scutes were clipped with microdissecting scissors (methods detailed in Lyman, 1990). A marked scute located one anterior to the vent denoted animals of litter 10; clipping scute five denoted litter 16. Scutes from position 11 on forward corresponded with the alphabetical subject code of each snake.

Snakes were also individually labelled for quick identification with head tags made from a light blue

plastic tape. Litter 10 was labeled with triangular, and litter 16 with square, tags bearing the letter code of the individual. Tags were marked using a waterproof marker. These head tags needed to be replaced after each skin molt.

Thamnophis sirtalis were placed in groups when 29 days old. Mixed species groups were formed at 44 days of age. Each group was housed in a 15 x 30 x 9 cm clear plastic vivarium with Aniboard© or artificial turf substrate, a fiberglass shelter and glass water dish. Each vivarium was visually isolated from all others with paper dividers. Isolated animals were maintained in original 13 x 18 x 5 cm vivaria. All group animals were fed separately in their individual containment boxes and allowed to rest there for approximately one hour before being returned to the group vivaria. All tanks were cleaned at the same time, about every two weeks or as needed.

Testing Schedule

Neonates were born June 27, 1988. Initial measurements and tests were performed prior to the first food offering at 14 days, and final testing was performed beginning at 121 days of age. These experiments were

completed when snakes were 238 days old. The testing schedule is detailed in Table 2.2.

Subject Mortality

Between 10 and 29 days of age 4 animals died. This resulted in a change of sample size between the initial and final Chemical and Site Choice tests. Deaths occurred at different times and did not seem to be related. Statistical analyses utilize only the animals that lived throughout the experimental period unless otherwise noted.

During the period from 158 to 218 days of age, another 13 animals died. Most of these animals died within a week of one another, and 11 of the 13 were worm fed animals. These deaths are probably the result of toxic worms, Lumbricus, purchased a few days earlier (described in Lyman, 1990). Deaths were almost exclusively among worm-fed snakes lab-wide, and necropsies were inconclusive, though intestinal blockage was suspected in some. Worms did not appear healthy, and may have been diseased, parasite-ridden, or accidentally fed something toxic before sale.

Table 2.2: Testing Schedule for T. sirtalis used in dissertation.

Days of Age	Test/Occurrence
1	First body measurements, sexed (N=67).
10	Initial Chemical Prey Preference Test (N=63).
12	Initial Feces-Based Site Choice Test (N=59).
14	Prey first offered (N=63).
25	Resexed (N=63).
29	Placed into housing groups (N=63).
91	Second body measurements (N=59).
121	Prey Surface-Based Site Choice Test (N=59).
135	Final Feces-Based Site Choice Test (N=59).
157	Final Chemical Prey Preference Test (N=59).
218	Arena Aggregation Tests (N=38).
237	Third body measurement (N=46), Experiments complete.

Note: Changes in N due to subject mortality except for Initial Feces-Based Site Choice Test, that excluded 4 animals due to small size, and Arena Aggregation that excluded animals housed with other species.

CHAPTER 3

GROWTH

Introduction

Since sexual maturity is generally size dependent (Porter, 1972) and capacity for growth diminishes with age after maturity (Andrews, 1982), it is important that net growth progresses relatively rapidly in young reptiles. Low growth rates in young reptiles are generally attributed to low prey availability. However, food quality also affects growth rates (Andrews, 1982), and different diets can have different energetic consequences (Godley, 1980).

Scudder-Davis and Burghardt (1987) demonstrated that common garter snakes, T. sirtalis, and plains garter snakes, T. radix, raised on diets of fish or earthworms showed different growth rates; animals fed fish grew at a greater rate than those fed worms. This work showed that the major difference between fish and earthworms as food items was the much higher calcium/phosphorus content in fish. Torrey (1971) cites calcium and phosphorus as being of crucial importance in formation of bone for growth in length. Accordingly, animals fed on fish grew faster than those fed worms despite the greater quantities of

earthworms eaten and equivalent calories between the two diets. In another study with T. radix (Burghardt, 1990; Burghardt and Lyman-Henley, in preparation) in which animals were raised on diets of fish or worm, controlled for amount and supplemented with calcium and phosphorus, different results were obtained. In this case, animals fed earthworm actually grew more rapidly than those fed fish, supporting the idea that calcium-phosphorus content is the most important difference in these foodstuffs.

Since differences in diet can have significant effects on both growth rate and responses to chemical prey cues in snakes, chemical prey preferences may also be correlated with growth rate. Since preferences for prey cues based on dietary experience is an important aspect of the current research program, it is necessary to control for differences in growth rate due to diet. In addition to the experiments described above, subjects were measured for growth rate while on strict diets of either earthworm or fish controlled for amount and calcium content. The effects on growth rate of sex, litter, and housing group were also analyzed.

In a concurrent study with Butler's garter snake, T. butleri, under the same research design (Lyman, 1990), diet (worms or fish) had no significant effect on growth, all differences being related to sex or litter. These results indicate that this feeding paradigm controls for

differences of nutritional value between the two diets. This analysis is here repeated with the T. sirtalis subjects of the present dissertation research.

Methods

Subjects

Subjects were 59 T. sirtalis during the first two observations and 46 during the last. In the last observation the total number of snakes was decreased by 13 due to subject mortality, as described in Chapter 2. Animals were derived from 2 litters (10 and 16) and maintenance is detailed in Chapter 2. Litter, sex, diet and housing information were outlined previously. In these observations, all test animals were available for the first two measurements. Table 3.1 summarizes subject groups and identifies individuals that died.

Diet and Feeding

Subjects were assigned to diets of fish (Gambusia affinis) or earthworm (Lumbricus terrestris) such that each regime was balanced as well as possible for sex, litter, size, and Initial Chemical Prey Preference (see Chapter 4). Prey animals and feeding protocol are detailed in Chapter 2. Food was provided twice weekly to all subjects. Any prey items not eaten within 2 hours

Table 3.1: Composition of groups of Thamnophis s. sirtalis (*) indicating animals that died between the second and third measurement observations.

			SUBJECT	
GROUP	DIET	LITTER	MALE	FEMALE
1	FISH	10	D, K	N, AB
		16	Y, AH	V, AF
2	FISH	10	S, V	Q, AD
		16	J, AE	S, AD
3	WORM	10	I*, X	Z, R*
		16	X, AC*	E, K
4	WORM	10	A*, F*	C*, AA*
		16	G, H	C, I*
5	FISH	10	L	M
		16	AB	R
	WORM	10	Y	P
		16	U	AJ
6	FISH	10	W	T*
		16	M	T
	WORM	10	AE*	B*
		16	W	AI*
(mixed species with T. butleri)				
10	FISH	10	-	H*, J
		16	B, Z	-
11	WORM	10	-	O, AC
		16	F, L	-
(individually housed animals)				
"12"	FISH	16	-	AA, P
	WORM	16	-	D

Note: Letters indicate subject I.D. codes.

were offered to the snake on forceps, which generally stimulated acceptance. Amounts of fish and worm meals were kept proportional, with a slightly greater mass of worm than fish being offered due to the greater water content of worms. A vitamin-mineral supplement was provided to all snakes twice monthly, and calcium-phosphorus supplements were given to worm-fed snakes at every third feeding. These measures were implemented based on information from Scudder-Davis and Burghardt (1987), and the earlier study with T. radix (Burghardt, 1990).

Toward the end of the observational period of the present study the quantities of available Gambusia decreased, necessitating a reduction in meal sizes (detailed in Chapter 2). Amounts of worm meals were kept proportional to the limited fish meals.

Procedure

Mass was recorded to the nearest 0.01 gram using a Sartorius electronic balance. Snout-vent lengths were measured to the nearest millimeter (mm) by gently stretching the animal along a meter stick. Snout-vent lengths are recorded from the tip of the rostrum to the posterior edge of the anal scale. Total lengths (from rostrum to tail tip) were not utilized. The day-of-birth measurements were taken by J. M. Schwartz and B. D. B.

Bowers. Subsequent measurements were taken by this author.

All subjects were measured three times: 1) on the day of birth, 2) when the subjects were 91 days of age, and 3) when 237 days of age. The first measurement occurred prior to any feeding, and subsequent measurements were taken at least 3 days after snakes had eaten in order to minimize effects of gut contents. Refer to the testing schedule as detailed in Chapter 2 for times of other experiments relative to measurements.

Statistical Analyses

Dependent measures snout-vent length (SVL) and mass (MS) were analyzed by ANOVA for main effects of diet, litter, sex and housing groups. Interaction effects were analyzed with 2-way ANOVAs excluding housing group. Differences between observations were analyzed with repeated measures ANOVAs. Multiple range tests were utilized in tests involving groups or observations. SVL and MS were also compared between animals that survived the duration of testing vs. those that died. Analyses were performed using Statgraphics 4.0 (STSC, 1990).

Snout-Vent Length (SVL)

SVL refers to absolute length in millimeters. Effects of diet, litter, and sex on SVL over observations are summarized in figure 3.1. Results of ANOVAs are summarized in table 3.2.

At the time of the initial observation of SVL at birth, there were significant differences between the two litters ($F(1,58)=35.56$, $p<0.0001$), such that snakes in litter 16 (mean SVL=157.5 mm) were longer in average SVL than snakes in litter 10 (mean SVL=144.3 mm). There were no significant differences due to sex or future diet or group.

At the second observation, litter again showed a significant effect ($F(1,58)=37.45$, $p<0.0001$) with litter 16 snakes (mean SVL=163.6 mm) being longer than litter 10 snakes (mean SVL=150.4 mm). Sex and group contributed no significant effects.

At the third observation litter was still significant ($F(1,45)=14.86$, $p=0.0005$) with snakes in litter 16 still longer (mean SVL=179.9 mm) than those in litter 10 (mean SVL=169.3 mm). There was also a significant interaction between litter and sex ($F(1,45)=9.69$, $p=0.004$). In this interaction, males were larger (mean SVL=181.5 mm) than females (mean SVL=177.9 mm) in litter 16 but smaller (mean

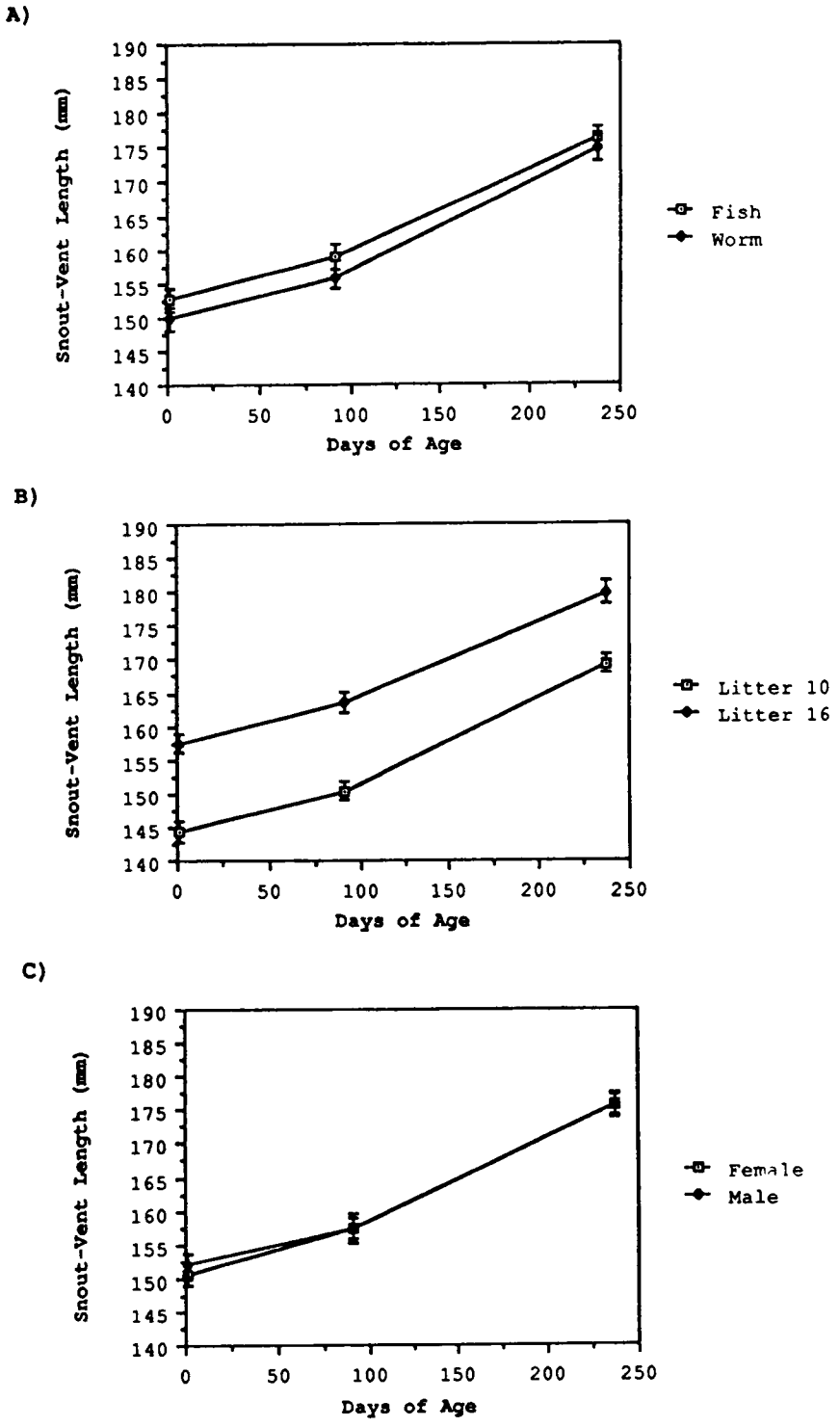


Figure 3.1: Snout-vent length (SVL) in mm ±1 standard error (SE) of T. sirtalis over time by A) diet, B) litter, and C) sex.

Table 3.2: Table of ANOVA results for snout-vent length (SVL) and mass (MS) of *T. sirtalis* over 3 measurements.

Variables	SVL F	(df)	p	MS F	(df)	p
Initial Measurement						
Diet	2.1	(1,58)	-	1.7	(1,58)	-
Litter	35.6	(1,58)	<.0001	1.8	(1,58)	-
Sex	0.03	(1,58)	-	1.0	(1,58)	-
Group	0.5	(8,58)	-	0.2	(8,58)	-
Second Measurement						
Diet	2.1	(1,58)	-	1.3	(1,58)	-
Litter	37.5	(1,58)	<.0001	10.7	(1,58)	.0021
Sex	0.4	(1,58)	-	1.8	(1,58)	-
Group	1.1	(8,58)	-	0.7	(8,58)	-
Diet x Litter				4.9	(1,58)	.032
Third Measurement						
Diet	2.0	(1,45)	-	0.7	(1,45)	-
Litter	14.87	(1,45)	.0005	7.9	(1,45)	.0086
Sex	0.0	(1,45)	-	2.8	(1,45)	.10
Group	1.1	(8,45)	-	0.9	(8,45)	-
Litter x Sex	9.69	(1,45)	.004			

Note: Values greater than p=.10 are denoted by "-." All main effects are listed, interactions are listed only if significantly occurring.

SVL=164.5 mm) than females (mean SVL=173.1 mm) in litter 10. There were no significant effects of diet, sex or group on final SVL.

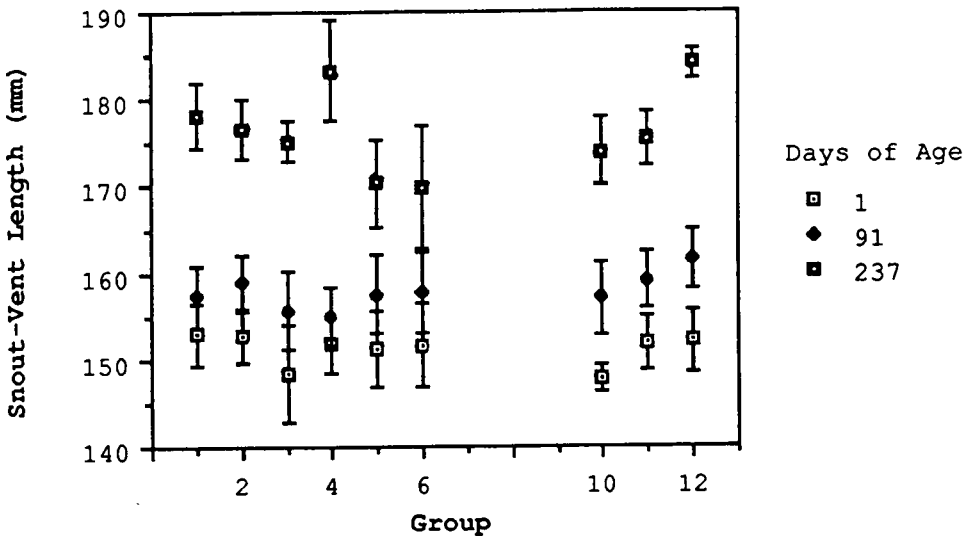
Although there were no significant effects of group upon SVL in any observation, the mean SVL of each housing group per measurement is presented in figure 3.2a for visual comparison. Animals in single diet groups (1-4) and individually housed (group 12) averaged slightly larger SVL by the final measurement than those snakes housed with animals on both diets (group 5 and 6) or with the second species (groups 10 and 11).

A repeated-measures ANOVA found that length increased significantly among observations ($F(2,163)=216.99$, $p<0.0001$). Of course this is not surprising as it just indicates that as the snakes grew older they also grew longer. The initial average SVL was 151.2 mm, the second mean SVL was 157.3 mm, and the final measurement averaged 175.7 mm, all three of which were significantly different from one another.

Mass (MS)

This measurement refers to the absolute mass in grams of each snake per observation. Figure 3.3 summarizes the effects of diet, litter, and sex on mass over observations. ANOVA results are described in table 3.2 (p.57).

A)



B)

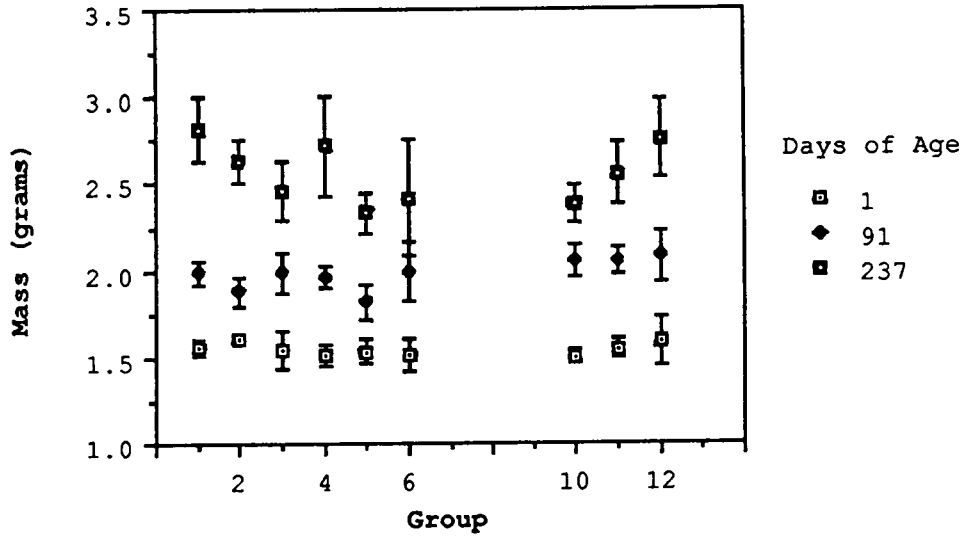


Figure 3.2: Comparison of mean sizes of animals by housing group over 3 observations. A) Snout-Vent Length (SVL) in mm ±1 standard error (SE), B) Mass (MS) in grams ±1 standard error (SE).

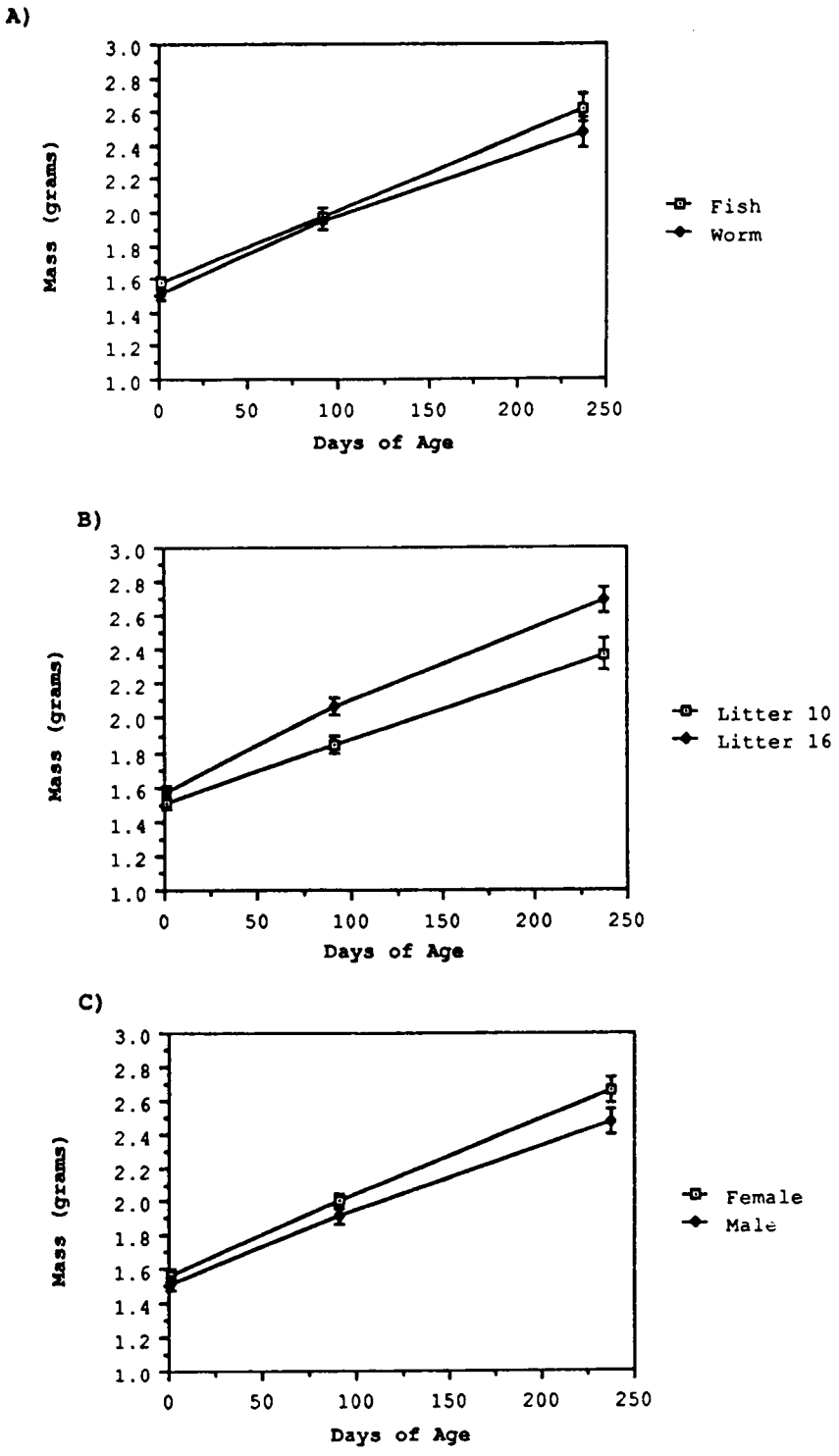


Figure 3.3: Mass (MS) in grams ±1 standard error (SE) of T. sirtalis over time by A) diet, B) litter, and C) sex.

At the time of the initial measurement, there were no significant effects of litter, sex, or future group or diet on MS.

At the second observation there was a significant effect of litter ($F(1,58)=10.74$, $p=0.002$) such that litter 16 snakes were heavier (mean MS=2.07 g) than litter 10 snakes (mean MS=1.85 g). There was also an interactive effect between litter and diet ($F(1,58)=4.93$, $p=0.032$). Worm-fed animals showed no difference between litters (mean litter 10 snakes MS=1.92 g, litter 16 snakes mean MS=1.97 g), but fish-fed animals did (litter 10 snakes mean MS=1.78 g, litter 16 snakes mean MS=2.15 g).

At the final measurement, there remained a significant difference between the litters ($F(1,45)=7.87$, $p=0.0086$) with animals in litter 16 (mean MS=2.69 g) outweighing those in litter 10 (mean MS=2.37 g). There were no other significant effects upon MS.

There were no significant effects of group upon SVL in any observation, however, the mean MS of each housing group per measurement is presented in figure 3.2b for visual comparison. As shown in the comparison with SVL, animals in single diet groups (1-4) and individually housed (group 12) averaged slightly larger SVL by the final measurement than those snakes housed with animals on both diets (group 5 and 6) or with the second species (groups 10 and 11).

Predictably, there was a highly significant increase in mass over the observations ($F(2,163)=240.12$, $p<0.0001$) initially averaging 1.54 g, having a mean MS at the second observation of 1.96 g, and at the final observation averaging 2.56 g. It should be noted that these weights were all very close, with only four tenths of a gram producing a significant difference between observation 1 and the following two.

Mortality

The animals that died between the second and third measurements were mostly on the worm diet (11 versus 2 fish-fed snakes), believed to be due to feeding on toxic Lumbricus (detailed in Chapter 2). Survivors and those that died did not differ significantly in either SVL or MS relative to diet. Mean SVL and MS are presented for each diet, litter and sex for snakes that survived or died in table 3.3.

Those that died were mostly from litter 10 (10 versus 3 from litter 16). There was a significant interaction of litter with mortality upon SVL at both the first ($F(1,58)=8.27$, $p=0.0064$) and second ($F(1,58)=7.43$, $p=0.0095$) measurements such that litter 16 animals that died were smaller (mean SVL 146.7 and 150.7 mm for 1st and 2nd measurements, respectively) than those that survived (mean SVL 158.6, 165.0 mm, respectively). There was also

Table 3.3: Comparison of mean sizes of *T. sirtalis* that died before the final measurement with those that survived, by litter, diet, and sex. A) Mean SVL and MS at first measurement (1 day of age), B) Mean SVL and MS at second measurement (91 days of age).

		Survived			Died		
		(N)	SVL	MS	(N)	SVL	MS
A) Initial Measurement (1 day of age)							
Litter	10	(18)	142.8	1.49	(10)	146.8	1.55
	16	(28)	158.6	1.61	(3)	146.7	1.23
Diet	Fish	(28)	152.9	1.58	(2)	148.5	1.49
	Worm	(18)	151.8	1.53	(11)	146.5	1.47
Sex	Female	(23)	151.1	1.58	(8)	148.8	1.52
	Male	(23)	153.8	1.55	(5)	143.6	1.41
Total		(46)	152.4	1.56	(13)	146.8	1.48
B) Second Measurement (91 days of age)							
Litter	10	(18)	149.9	1.78	(10)	151.3	1.97
	16	(28)	165.0	2.09	(3)	150.7	1.8
Diet	Fish	(28)	159.3	1.98	(2)	154.0	1.97
	Worm	(18)	158.8	1.96	(11)	150.6	1.93
Sex	Female	(23)	158.6	1.99	(8)	153.5	2.00
	Male	(23)	159.6	1.95	(5)	147.4	1.82
Total		(46)	159.1	1.97	(13)	151.2	1.93

a significant interaction of litter with mortality at the first measurement ($F(1,58)=12.04$, $p=0.0013$), and a non-significant interaction at the second measurement ($F(1,58)=3.51$, $p\approx0.06$), which show the same pattern of litter 16 snakes that died averaging lower MS than survivors.

However, this difference appears to be due to a single very small individual that died. This animal, 16AC (a worm-fed male from group 3), had an initial SVL of 127 mm and MS of 0.8 g, and a second (final) SVL of 132 mm and MS of 1.17 g; quite small compared to the rest of its litter. On the other hand, the smallest animal in litter 10 (10W, fish-fed male in group 6, at 121 mm initial SVL and 1.03 g initial MS) survived, leading to litter 10 survivors actually averaging slightly smaller than those that died. There were no differential effects of sex upon mortality.

Discussion

The main difference in both mass and snout-vent length is between the litters, with litter 16 snakes being longer and heavier than litter 10 animals throughout the study. Intrinsic, heritable factors affecting body size and composition are most likely the basis of this difference, and are therefore not under the direct influence of maintenance measures (Andrews, 1982). It is interesting

to note that even regarding mortality, the only difference found was between litters, with litter 10 animals apparently being more susceptible to the toxicity of the earthworms they ate than the larger litter 16 snakes.

The lack of strong effects of diet type upon growth in these animals is welcome for the purposes of the present research. Compared to previous work with T. sirtalis and T. radix where animals fed fish grew at a greater rate than those fed worms (Scudder-Davis and Burghardt, 1987), it seems that the addition of calcium-phosphorus supplements to the worm diet negated any deficiency of these minerals compared to that contained in fish (Burghardt, 1990). The maintenance regime used here with T. sirtalis had also virtually negated differential effects of diet on growth in T. butleri (Lyman, 1990), indicating that its success is applicable to more than just one species.

Crews et al. (1985) demonstrated that female T. sirtalis parietalis were larger than males within 3 weeks of birth, due to inhibitory effects of androgens on growth. The only effect of sex found in the present study with T. sirtalis, however, was the interactive effect with litter upon snout-vent length at the final measurement. In T. butleri (Lyman, 1990) the main difference in growth between animals raised in the same maintenance regime was dependent on sex, such that males were consistently larger

than females. It was argued that the constraints on meal size may have not allowed females to grow to their full potential. With the current T. sirtalis, there may be a similar, though weaker effect, that kept females from outgrowing males as had been previously found for this species. Alternatively, this subspecies may not normally show the same sexually dimorphic growth rate as their northern cousins. It is also worth noting that Crews et al. (1985) kept their animals in group-housing situations, separated by sex and age, but apparently fed the snakes while in these groups (on a diet of canned fish with vitamin-mineral supplements). This methodology can result in a wide variation of growth rates due to competitive exclusion between animals such that a few individuals consume a far greater amount of food than others (Lyman-Henley, unpublished data; Schwartz, pers. comm.; Wilbur, 1977).

Another factor noted in Lyman (1990) regarding T. butleri dealt with the rate of growth between different measurements. In that species, growth was curtailed between the second and last measurements, with some animals actually losing weight. This was mainly attributed to the decrease in available food toward the end of the study, with a possible effect of handling stress or circannual rhythms. The T. sirtalis in the current study were subject to the same deprivations, yet

did not show this decrease in growth, despite being larger animals. Growth actually occurred at a slightly greater rate between the second and last measurements than between the first two in this species. Perhaps this generalist species is better able to assimilate small meals than the earthworm specialist T. butleri.

In conclusion, it appears that effects of diet on growth rate were minimal. Virtually all effects on growth were based on litter. If it can be assumed that health and its effects on responsiveness are correlated with growth rate in neonate snakes, this study, especially compared with the comparable results found for T. butleri (Lyman, 1990), demonstrates that it is possible to control for this in animals on different diets for experimental purposes. The feeding regimes of fish or calcium-supplemented worms seem to produce only minor differences in growth rate of neonate T. sirtalis. This lack of effect of different diets on growth should indicate negligible differences in snake condition which might have impacted other measures used in this research.

CHAPTER 4

CHEMICAL PREY PREFERENCES

Introduction

Neonate garter snakes respond to aqueous surface extracts of certain prey species without prior experience and show differing levels of responsiveness to varying prey species (Burghardt, 1969). This is accomplished by the vomeronasal system, comprised of the vomeronasal organ (VNO), located in the roof of the snake's mouth, and the forked tongue (Burghardt, 1966, 1967; Burghardt and Pruitt, 1975; Halpern, 1987; Halpern and Kubie, 1983; Wilde, 1938). Tongue-flick rates have been shown to correspond to a snake's activity level (Chiszar and Carter, 1975; Gove and Burghardt, 1983) and increase in the vicinity of natural prey items (Burghardt and Denny, 1983; Carr and Gregory, 1976; Chiszar, Scudder and Knight, 1976). This allows the use of measures of interest such as the Tongue-Flick Attack Score (TFAS), which scores responsiveness to chemical prey cues based on tongue-flick rate and latency to attack (Burghardt, 1969; Cooper and Burghardt, 1990).

Chemosensory prey preferences of snakes in different populations have been demonstrated to have a genetic basis

(Arnold 1977, 1981) without influence of maternal diet (Burghardt, 1971). Different individuals within a litter may also show preferences for different prey types, a phenomenon known as "prey preference polymorphism" (Burghardt, 1975).

Garter snakes can develop the ability to discriminate between different species of the same prey types (Fuchs and Burghardt, 1971). Experience with various prey can affect changes in the initial preferences and discriminative abilities of snakes. Eastern garter snakes, T. sirtalis, fed exclusively on fish exhibited higher responses to chemical extracts of fish than did animals raised on frogs (Arnold, 1978). Recent studies demonstrate that experience with prey also enhances the ability of snakes to discriminate increasingly dilute concentrations of prey surface extracts (Burghardt, 1990; Burghardt and Lyman-Henley, in preparation).

The plains garter snake, T. radix, a generalist feeder known to eat both worms and fish in the wild, has been shown to exhibit such response shifts in chemical prey preference based on experience. Animals in the same litter raised on either earthworm or fish diets shifted preference towards the prey type experienced regardless of initial response (Burghardt, 1990; Burghardt and Lyman-Henley, in preparation).

Thamnophis butleri, a close relative of T. radix, in similar testing situations showed a striking effect of experience upon prey preference (Lyman, 1990). Thamnophis butleri is a species which specializes on earthworms and is not known to feed on fish in the wild. Animals from two litters showed a strong preference for worm surface extracts prior to experience with prey and barely discriminated fish extract from water. Snakes fed the "natural" diet of worms exhibited no change in this preference. However, those fed on fish, although still showing a high response to worm cues, showed an even higher response to the familiar, though "unnatural" fish prey cues.

In this chapter I present the results of an experiment replicating and refining previous work regarding the effects of experience upon chemical prey preferences in garter snakes. Thamnophis sirtalis is, as described in Chapter 2, a dietary generalist, feeding on a wide variety of prey species (Carpenter, 1952a; Conant and Collins, 1991; Fitch, 1965; Lagler and Salyer, 1945). In the following experiment, 59 T. sirtalis were tested for chemical prey preference before and after a relatively extended period of experience with prey (either earthworms or fish) to investigate how such experience may affect the responses of a feeding generalist.

Previous work suggests that experience should shift initial preferences towards the familiar prey (Arnold, 1978; Burghardt, 1990; Lyman, 1990). It is suspected that similar feeding habits will confer similar effects of experience upon prey preferences. Therefore, this shift in preference is not expected to be as dramatic as that of the specialist T. butleri, but more similar to that of the generalist T. radix, due to a greater natural flexibility in prey acceptance.

Two specific hypotheses as introduced in the introduction can therefore be tested:

1. Thamnophis sirtalis will respond to both worm and fish extracts without prior experience, as both are part of the natural diet of the species.

2. After experience with prey, chemical prey preferences will shift in favor of the familiar prey type.

In addition, details regarding the initial prey preferences and effects of dietary experience upon members of this specific population of a widely ranging species will be provided. These data will then provide a basis of comparison for the remainder of the experiments dealt with in this dissertation.

Methods

Subjects

Subjects were 59 *T. sirtalis* from 2 litters (88-MI-1610 and 88-MI-1616). Although 63 snakes were included in the initial test, only those that survived for the duration of data collection are discussed. Subjects were assigned to diets of nightcrawler, *Lumbricus terrestris*, or mosquito fish, *Gambusia affinis*, such that each regime was balanced as well as possible for sex, litter, size, and Initial Chemical Prey Preference (described below). Maintenance is detailed in Chapter 2.

Preparation of Stimuli

Stimuli consisted of surface extracts of the two prey types, nightcrawler or mosquito fish, and a control of de-ionized water. Surface extracts of prey items were prepared by placing 3.0 g of clean, dry prey into 10 ml of 60°C distilled water and stirring gently for 2 minutes (Burghardt, 1969). The liquid produced is centrifuged at 2500 rpm for 10 minutes, the clear supernatant then being frozen in sealed glass vials until use.

Extract Test Procedure

Animals were removed from their groups and placed in testing containers the afternoon before testing to allow

time to acclimate to their surroundings. Approximately half of the snakes were tested on each of two consecutive days due to space constraints, with groups balanced for litter, sex, and in the final test, diet. All snakes were visually isolated from each other at all times with paper dividers. Before testing began, water dishes were removed for the duration of testing. Shelters remained in the containers unless temporary removal was necessary to access an individual.

Testing consisted of presentation of a cotton swab dipped in surface extracts of prey or a water control to each snake for 30 seconds. The number of tongue flicks (both total, and those directed at the swab) and latency to strike in case of predatory attack were then recorded. The swab was held slightly to one side of the subject's snout at a distance of approximately 1 to 2 cm, allowing determination of whether the animal was directing its tongue-flicks toward the stimulus or not. If no response was made within 10 seconds, the snake was touched lightly on the lips with the swab. Swabs that touched any part of a snake or its tank during testing were discarded. Otherwise, swabs were freshened in extract between subjects and reused. Each stimulus was presented to all snakes once before the next presentation round was begun.

Initial Test

Thamnophis sirtalis were initially tested for chemical prey preferance at 10 days of age. There were 63 total subjects tested, 30 from litter 10 and 33 from litter 16.

All animals were tested in their home containers, which were 13 x 18 x 5 cm plastic gel boxes with Aniboard substrate (see also Chapter 2). The temperature in the room was 24°C, the humidity 42%.

Testing began at 0900 hrs, and the time between presentation of stimuli was approximately 45 minutes. Due to constraints imposed by the feeding schedule of the subjects, prey stimuli were presented only once each, in the order: Control, Worm, Fish, Control. This order had been used in previous work (Lyman, 1990; Schwartz, 1989) and was adhered to for ease of comparison of data.

Final Test

Thamnophis sirtalis were retested for chemical prey preferences at 157 days of age. At this time there were 59 surviving subjects, 28 in litter 10 (14 fed fish, 14 fed worms) and 31 in litter 16 (16 fed fish, 15 fed worms).

Burghardt (1969) demonstrated that responses of naive snakes to water controls offered first in a series tend to be higher than later presentations (a "novelty" effect).

Responses to control presentations are also higher following an extract eliciting an attack than those following an ineffective stimulus extract (a "carry-over" effect). In order to control for these order effects, stimulus extracts were presented twice in one of two orders: order 1 - "control, worm, fish, control, fish, worm;" or order 2 - "control, fish, worm, control, worm, fish." Animals tested with different stimulus orders were balanced for diet, sex and litter.

Animals had not eaten for one week prior to testing, the maximum between-feeding period. Animals were individually tested in 15 x 30 x 9 cm clear plastic containers with a butcher-paper substrate. Container size was increased since the initial test due to the growth of the subjects. Test schedule and procedure was otherwise identical to that of the initial test. Temperature in the test room was 24°C, humidity was 44%.

Data Analysis

TFAS: The primary measure used to analyze results was that of Tongue-Flick Attack Score (TFAS) which is based on the number of tongue flicks directed at the stimulus adjusted by latency of attack. TFAS is derived by the equation:

$$TFAS = TFmax + (Trial\ Length - Latency)$$

where "TFmax" is the highest number of tongue flicks directed to the swab by any snake in a given test. In this way, attacks count more heavily than tongue flicks in determining interest in the stimulus presented (Burghardt, 1969; Cooper and Burghardt, 1990).

TFAS scores were log-transformed (natural log + 1) for all analyses. These transformed scores will be referred to as "lnTFAS."

Two-way ANOVAs were utilized to determine effects and interactions of litter and sex for each test within each dietary regime. For the final test, data from the first three stimulus presentations to each snake were analyzed, and the two orders of presentation were compared. Repeated measures ANOVAs were performed between tests as well as among stimuli for animals on each diet. They were also performed for each order of stimulus presentation, as well as for overall effects. The frequency of attack to the various stimuli was also considered.

F-W Score: A difference score, "Fish - Worm" (F-W), was computed by subtracting the mean TFAS for worm from the mean TFAS for fish for each animal. This value reflects the difference in response of an animal to the two prey stimuli, and can be viewed as a measure of the direction of the subject's prey preference.

Effects of diet, litter, sex and housing group were determined per test and diet with four-way ANOVAs. Repeated measures ANOVAs were performed to test for effects between initial and final tests for all animals and those on each diet. Multiple Range tests were used to establish the location of significant differences within the ANOVAs. All analyses were performed using Statgraphics 4.0 (STSC, 1990).

Preference: Animals were also scored for preferences in favor of earthworm or fish cues based on the F-W Score, such that positive values reflect a stronger response toward fish and negative values a response biased toward worm. The numbers of animals of each diet, litter, and sex that showed preferences for each prey type could thereby be determined. Chi-square contingency tests were performed on these data. Changes in preference between initial and final prey preference tests were analyzed using the McNemar test for the significance of changes (Siegel and Castellan, 1988).

Results

Initial Test

TFAS: There was a significant difference in response to the stimuli ($F(3,235)=24.63$, $p<0.0001$), with lnTFAS

toward the water controls averaging significantly lower (mean lnTFAS=2.0) than that toward either fish (mean lnTFAS=2.62) or worm extract (mean lnTFAS=2.83). Responses to the two prey extracts did not differ significantly from each other (Figure 4.1). There were no significant effects of sex, assigned diet, or assigned group in this portion of the experiment.

Only 16 attacks were made, 13 of which were toward the worm stimulus (Chi-square(1)=6.25, $p<0.02$). This indicates that although snakes readily investigated both prey extracts (as indicated by TFAS), they were more likely to accept the worm cues as indicative of acceptable prey.

F-W Score: Responses to the two prey stimuli were significantly different between litters ($F(1,58)=7.18$, $p=0.01$), with litter 10 showing a stronger worm bias (mean F-W score= -13.96) than litter 16 (mean F-W score= -0.39). Overall, the responses reflected a general bias in favor of worm stimuli with an average F-W Score of -6.83. Again, there were no significant effects of sex, assigned diet, or assigned group.

Preference: There were no significant effects of litter, sex, or assigned diet upon the frequency of preferences displayed toward the two prey extracts, although more animals showed a worm preference. This is

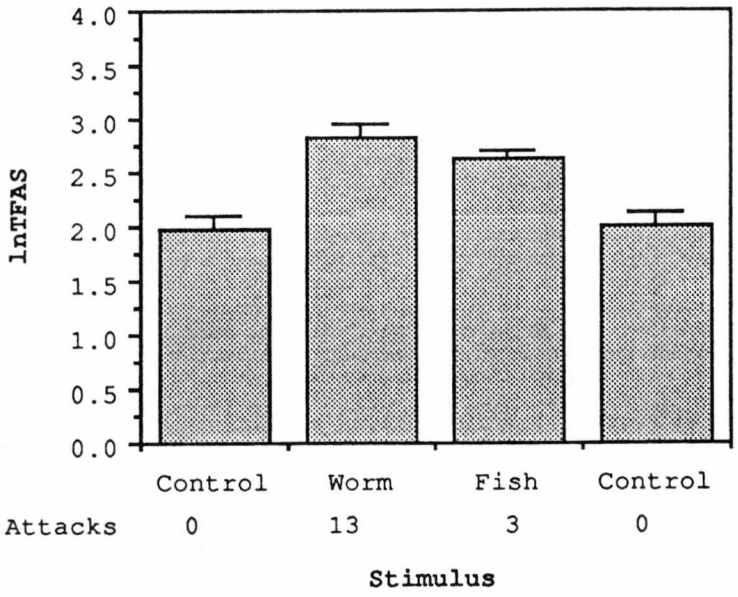


Figure 4.1: Log-transformed Tongue Flick Attack Score (lnTFAS) + 1 standard error (SE) results of Initial Chemical Prey Preference Test of Thamnophis sirtalis using surface extracts of worm (Lumbricus), fish (Gambusia) and water control.

summarized in Table 4.1. However, animals having worm preferences tended to have greater difference values (maximum F-W score -54.7) than those preferring fish (maximum F-W Score 30.8). This reflects the greater number of attacks directed at worm cues, and a generally greater strength of worm preferences.

Final Test

TFAS- Diet effects: Responses to fish and worm stimuli were significantly higher than those toward water controls, though there was no difference between the responses toward prey types. For order 1 (CWFCFW) animals fed fish showed this significant difference in response to the stimuli presented ($F(5,89)=29.7$, $p<0.0001$), as did those on the worm diet ($F(5,83)=48.4$, $p<0.0001$). In the fish-fed animals, the familiar prey did elicit slightly higher responses than the unfamiliar, but worm fed animals showed no mean difference. Also, there was virtually no difference in attack rate, only two fewer strikes given to unfamiliar prey by worm fed animals than other conditions. Figure 4.2 details these results.

Responses to order 2 stimuli (CFWCWF) were also significant for both animals fed fish ($F(5,89)=23.82$, $p<0.0001$) and those fed worms ($F(5,89)=10.04$, $p<0.0001$). Here, the familiar prey did tend to have a slightly greater mean lnTFAS and a greater number of attacks than

Table 4.1: Number of T. sirtalis prior to feeding experience showing chemical prey preferences toward fish or earthworm.

Variable		N	Preference: Fish	Worm	None	Significance
Assigned Diet:	Fish	30	11	18	1	n.s
	Worm	29	14	15	0	
Litter:	10	28	11	17	0	n.s
	16	31	14	16	1	
Sex:	Male	28	10	17	1	n.s
	Female	31	15	16	0	
Total:		59	25	33	1	n.s.

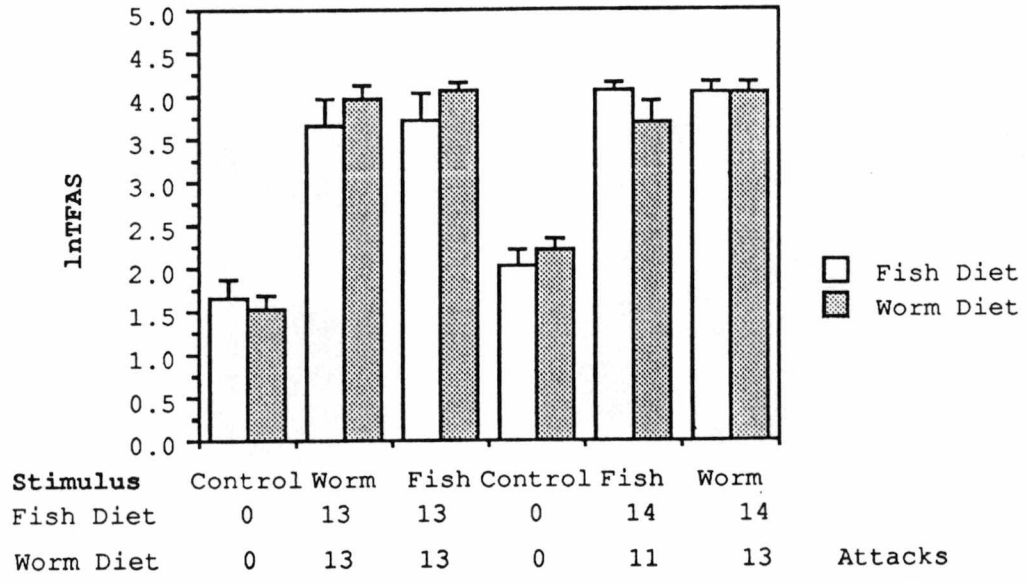


Figure 4.2: Log Tongue Flick Attack Score (lnTFAS) +1 standard error (SE) responses of T. sirtalis on different diets during Final Chemical Prey Preference Test to stimuli presented in order 1: control (water), worm, fish, control, fish, worm.

unfamiliar in both cases, though again, significant differences were only between responses to control vs. prey stimuli. These results can be seen in Figure 4.3.

TFAS- Group effects: For stimulus presentation order 2 (CFWCWF) there was an effect of housing group, significant for both worm fed animals ($F(5,89)=10.3$, $p<0.0001$) and fish fed animals ($F(5,89)=7.1$, $p<0.001$). Higher mean lnTFAS were generally delivered toward stimuli that matched the diet that group was fed. However, groups which housed animals on both diets (groups 5 and 6) differed in that group 5 overall preferred worm cues while group 6 preferred fish cues, regardless of individual diets. Animals that were maintained in isolation rather than group housing situations were generally less responsive to stimuli (Table 4.2). The loss of statistical power combined with negligible changes of mean lnTFAS did not warrant analyses utilizing only groups 1 through 4 (groups of animals exposed only to one diet and species) rather than all groups of animals pooled.

TFAS- Litter effects: Examining only the results of the first three stimulus presentations, there was a significant effect of litter in order 2 (CFW) ($F(1,89)=7.03$, $p=0.0098$) such that litter 10 snakes were more responsive (mean lnTFAS over all stimuli= 3.09) than

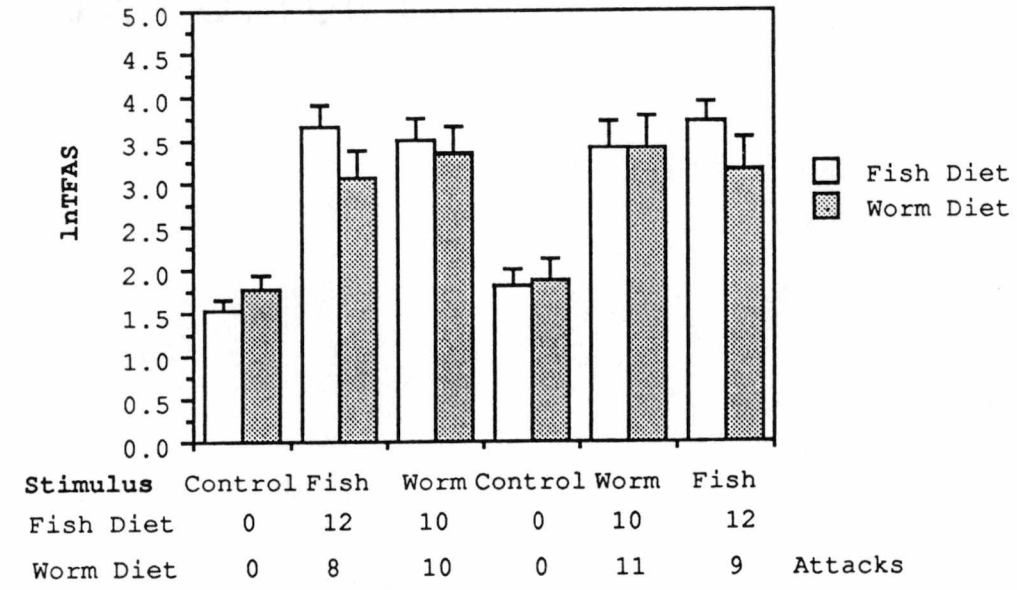


Figure 4.3: Log Tongue Flick Attack Score (lnTFAS) + 1 standard error (SE) responses of T. sirtalis on different diets during Final Chemical Prey Preference Test to stimuli presented in order 2: control (water), fish, worm, control, worm, fish.

Table 4.2: Mean lnTFAS (and Standard Error, SE) of *T. sirtalis* in different housing groups toward chemical stimuli in Final Chemical Prey Preference Test.

Group	Diet	N	Stimulus: Control	Worm	Fish
1	F	8	1.90 (0.14)	3.49 (0.24)	**3.63** (0.28)
2	F	8	1.73 (1.68)	3.77 (0.28)	**3.85** (0.20)
3	W	8	2.00 (0.12)	**4.07** (0.04)	3.71 (0.20)
4	W	8	1.80 (0.19)	**3.43** (0.27)	3.34 (0.27)
5a	F	4	1.53 (0.37)	**4.12** (0.04)	3.96 (0.02)
5b	W	4	2.00 (0.16)	**4.12** (0.05)	3.17 (0.39)
6a	F	4	1.98 (0.19)	3.62 (0.37)	**4.18** (0.02)
6b	W	4	2.09 (0.30)	3.91 (0.28)	**4.20** (0.01)
10	F	4	1.82 (0.14)	3.77 (0.28)	**4.03** (0.09)
11	W	4	1.43 (0.29)	**3.66** (0.35)	3.51 (0.51)
12a	F	2	1.02 (0.42)	2.54 (0.82)	**2.67** (0.82)
12b	W	1	**1.24** (1.24)	0.00 (0.00)	0.97 (0.97)

Note: Highest mean lnTFAS for group is underlined.
Abbreviations: F = fish diet, W = worm diet.

litter 16 animals (mean overall lnTFAS= 2.55). Among worm fed animals, an interaction effect between order and litter ($F(1,89)=5.3$, $p=0.024$) showed animals in litter 16 to be slightly more responsive than litter 10 snakes in order 1 (mean overall lnTFAS= 3.27 and 3.09 respectively), but much lower than litter 10 snakes in order 2 (mean overall lnTFAS= 2.46 and 3.02 respectively).

TFAS- Sex effects: There is a significant interaction between order and sex for fish-fed snakes ($F(1,89)= 4.1$, $p=0.046$) where females show higher responses in order 1 (mean lnTFAS =3.15) than 2 (mean lnTFAS= 2.69) whereas males show higher responses in order 2 (mean lnTFAS= 3.00) than order 1 (mean lnTFAS= 2.64). There were no significant interactive effects of sex and stimulus presentation in order 1 ($F(2,86)= 2.4$, $p=0.09$), but females showed a higher response to worm (mean lnTFAS= 4.11) than fish (mean lnTFAS= 3.75), whereas males showed a higher response to fish (mean lnTFAS= 4.02) than worm (mean lnTFAS= 3.51).

F-W Score- Diet effects: Effects of diet upon F-W Score only showed a significant interaction with litter and sex, and are discussed under those headings below.

F-W Score- Group effects: No effects of group were found in F-W Scores, past a general pattern of matching diet.

F-W Score- Litter effects: There was a significant difference in F-W score between animals of the two litters ($F(1,58)=9.7$, $p=0.0032$). Overall, litter 10 animals preferred worm (mean F-W score= -11.02) and litter 16 animals preferred fish (mean F-W score= 7.12). Among fish fed animals this is also seen ($F(1,29)= 8.4$, $p=0.0086$) such that litter 16 snakes showed a strong preference for the familiar prey (mean F-W score = 13.47) whereas litter 10 snakes still preferred the worm stimulus (mean F-W score= -7.98). This is shown in Figure 4.4.

F-W Score- Sex effects: Though not significant, there was an effect of sex ($F(1,58)= 3.7$, $p=0.061$) such that females preferred worm (mean F-W score= -7.24) while males preferred fish (mean F-W score= 4.87). Among worm eaters this can still be seen ($F(1,28)= 3.89$, $p=0.063$) where females prefer worms (mean F-W score= -14.9) and males still show a slight preference for fish (mean F-W score= 2.28). This is shown in Figure 4.5.

Preference: There was a significant effect of litter (Chi-square=8.02, $p=0.0046$) such that litter 10 animals

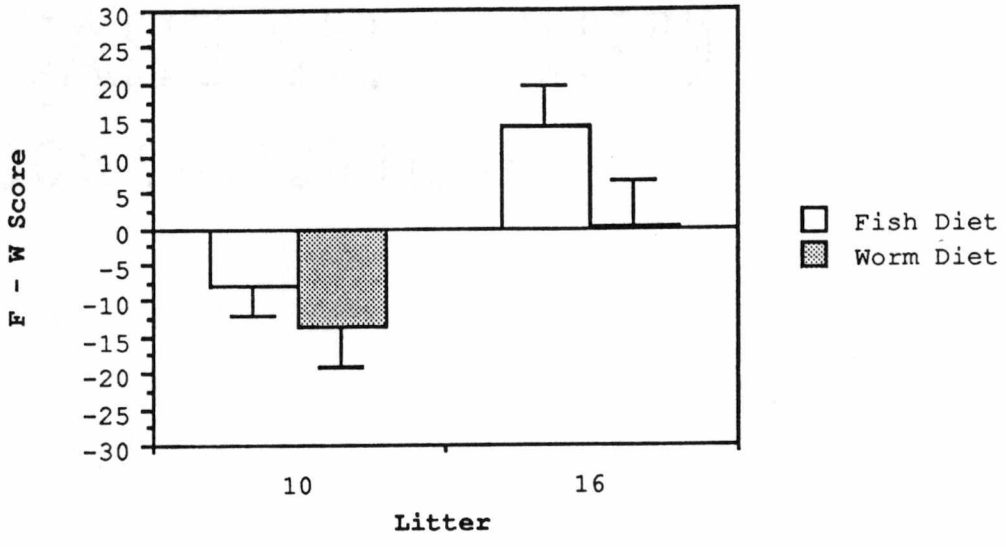


Figure 4.4: Fish - Worm responses (F-W Score) ±1 standard error (SE) of two litters of T. sirtalis on different diets in the Final Chemical Prey Preference Test.

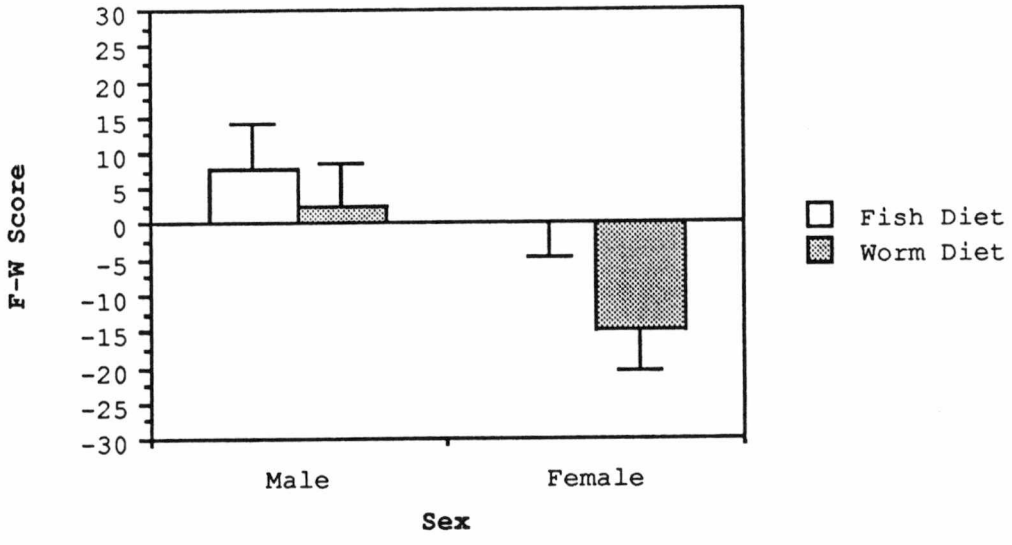


Figure 4.5: Fish - Worm responses (F-W Score) with 1 standard error (SE) of T. sirtalis on different diets by sex in the Final Chemical Prey Preference Test.

more frequently preferred worm, whereas litter 16 animals preferred fish. There was also a significant effect of sex (Chi-square=3.85, p=0.049) in which females more often preferred worm whereas males tended to prefer fish. Although not significant, animals tended to prefer the familiar prey (Chi-square=2.95, p=0.086). All preferences are summarized in Table 4.3.

Comparisons Between Tests

TFAS: Repeated measures ANOVAs found a significant difference in overall lnTFAS between the initial and final tests ($F(1,589)=52.6$, $p<0.0001$) due to a general increase in responsivity toward the stimuli. This change was significant for both the worm-fed animals ($F(1,289)=23.8$, $p<0.0001$), and those fed fish ($F(1,299)=28.8$, $p<0.0001$).

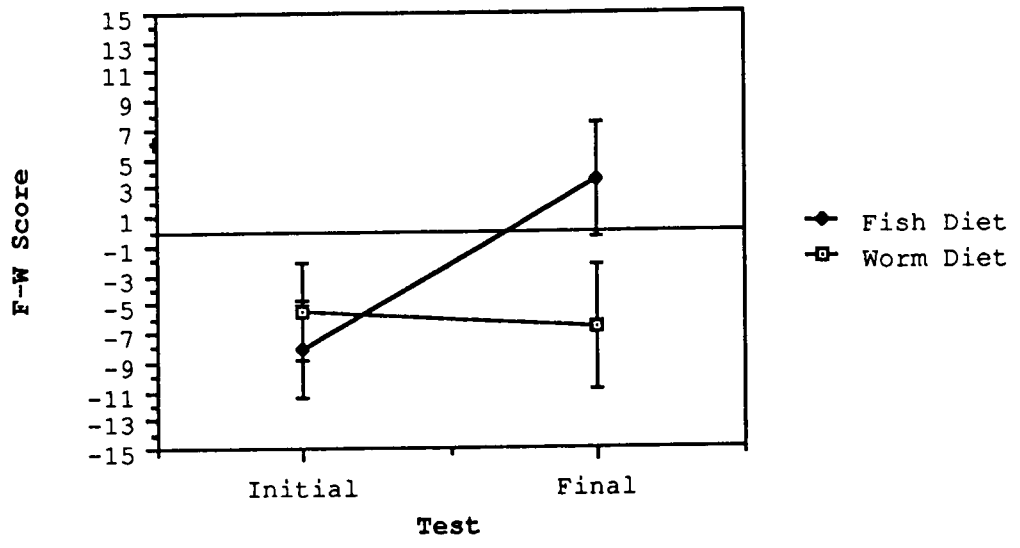
F-W Score: Repeated measures ANOVA also found a significant difference in F-W score of fish fed animals between the two tests ($F(1,59)= 6.75$, $p=0.0145$), with the mean response moving from a fairly strong worm bias (mean F-W score= -8.09) to a weak fish bias (mean F-W score= 3.46). Worm fed animals showed no change of preference between tests (mean F-W scores= -5.5 and -6.6 respectively), and the overall change was not significant (mean F-W scores= -6.8 to -1.49). This is illustrated in Figure 4.6a. When analyzed by litter, only fish-fed

Table 4.3: Number of T. sirtalis after feeding experience showing chemical prey preferences for surface extracts of fish or earthworm.

Variable		N	Preference: Fish	Worm	None	Significance
Diet:	Fish	30	16	12	2	p=0.086
	Worm	29	10	19	0	
Litter:	10	28	7	20	1	p=0.0046**
	16	31	19	11	1	
Sex:	Male	28	16	11	1	p=0.049*
	Female	31	16	20	1	
Total:		59	26	31	2	n.s.

Note: "*" denotes significant chi-square (3.85, df=1).
"**" denotes significant chi-square (8.02, df=1).

A)



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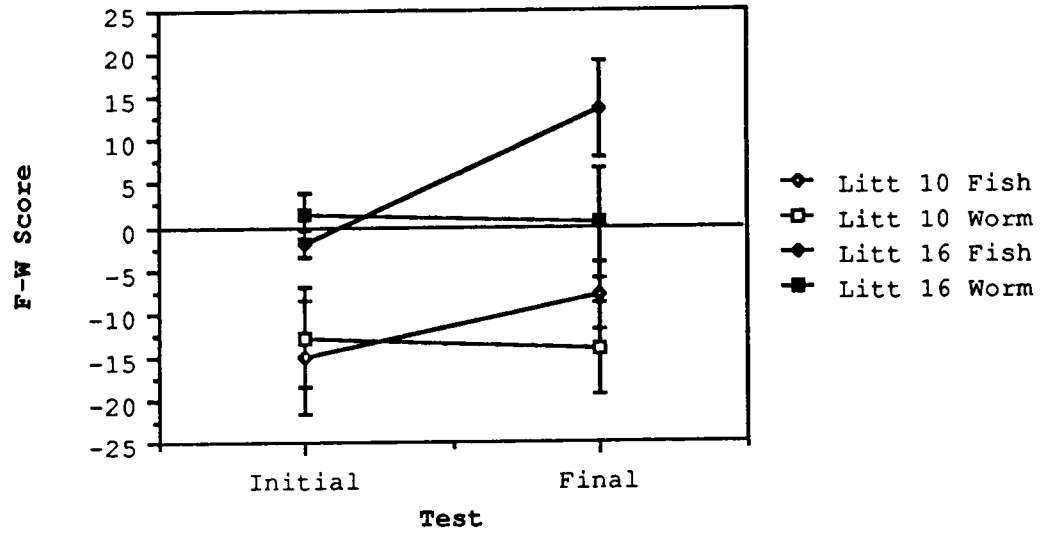


Figure 4.6: Fish - Worm responses (F-W Score) ±1 standard error (SE) of T. sirtalis on different diets between Initial and Final Chemical Prey Preference Tests. A) All snakes, B) Separated by litter.

litter 16 snakes showed a significant change in preference ($F(1,31)=7.64$, $p=0.015$), moving from a weak worm (mean F-W score= -2.0) to a strong fish bias (mean F-W score= 13.5) between tests (Figure 4.6b). Overall, 33 of the 59 snakes showed a change in F-W Score in favor of the familiar prey.

Preference: A McNemar test of change found a significant change of preference frequency in favor of familiar prey after experience (Chi-square=4.8, $p<0.02$), as illustrated in Table 4.4a. This analysis utilizes only those responses that change between tests, therefore data are here presented as a preference for "assigned" or "other" diet rather than fish or worm to allow a more direct comparison of results. Although not significant, both worm and fish-fed animals more frequently shifted preference in favor of assigned prey than not. These results can be seen in Table 4.4b.

Discussion

Prior to feeding experience, the T. sirtalis in this experiment gave high responses to both fish and worm aqueous surface stimuli over a water control. Since both prey extracts elicited high frequencies of tongue-flicking, a preference for the worm is revealed only by

Table 4.4: Changes in chemical prey preference of *T. sirtalis* between initial and final tests. A) Number of total subjects responding to the "other" and "assigned" diets over tests; B) Responses of subjects in each feeding regime.

Final Test Preference	Initial Test Preference: Other	Initial Test Preference: Assigned	Significance
A) All Subjects			
Assigned	21	16	$p<0.02$*
Other	12	9	
B) Fish Diet			
Assigned	12	6	n.s.
Other	7	5	
Worm Diet			
Assigned	9	10	n.s.
Other	5	4	

Note: "*" denotes significant McNemar chi-square (4.8, df=1).

the much higher rate of attack toward that stimulus. This interest in both prey, with a greater tendency to attack worm, is in keeping with data regarding the natural prey of T. sirtalis from Michigan populations.

There is only a weak effect of experience on prey preference demonstrated in the experiments here described. The worm specialist, T. butleri, in similar testing showed a dramatic effect such that animals fed fish (a prey not taken in the wild) showed strong responses in favor of that prey after experience, though maintaining a strong response to the natural worm prey (that had never been experienced). Animals that were not fed fish showed only a weak response to fish cues, if at all. The generalist, T. sirtalis, exhibited a strong response to both prey, but did show influences of experience since fish-fed animals moved from a moderate worm preference to a weak fish bias, although worm-fed animals showed no change in bias.

However, this weakness of differences in preference seems partly to be due to a masking effect of litter. There was a difference between the litters in response to prey chemical cues from the outset, such that litter 10 snakes showed a strong preference for worm cues while litter 16 animals were ambivalent. This finding may be related also to the different responsiveness of the animals of the two litters in the initial test, as 15 of the 16 attacks made in the first test were given by snakes

from litter 10. In the final test, animals in different litters still showed great differences in preference, with litter 16 fish-eaters now demonstrating a strong preference for fish cues, while litter 10 snakes retained a worm preference. These snakes also showed some effects of sex on preferences such that females showed greater preferences for worm while males demonstrated more of a fish bias, even in worm fed animals.

It may be that such an array of variations at the level of litter and sex confers an adaptive advantage by reducing competition between snakes with slight differences in prey preference within the broad range of prey a generalist species such as T. sirtalis feeds upon. It also fits well with the ability of T. sirtalis throughout its range to coexist with various other species more specialized in one prey or another. Such flexibility in the prey preferences of T. sirtalis will enable some individuals to adjust to available dietary niches opposite a wide array of competitors.

It is also worth noting the basic pattern of response to stimuli shown by animals in different housing groups. For 6 of the 9 possible housing situations, all animals in a group ate the same prey, and mean responses were highest for that stimulus. However, the 2 groups that contained animals on each diet differed from this pattern. Mean response was higher for worm stimuli in group 5 and fish

stimuli in group 6, regardless of individual diets. This may be weak evidence that there could be some influence of the diets of familiar conspecifics upon prey preference external to one's own experience, since snakes in these groups could be sources of chemical cues (in feces or on skin) of the unfamiliar prey.

In conclusion, T. sirtalis from Michigan showed an initial slight preference to worm surface cues, which shifted weakly in favor of familiar prey cues after experience. Snakes showed high responses to both prey stimuli regardless of experience, which corresponds to their being generalist feeders. There were pronounced litter effects from the outset on both responsiveness and preference, including likelihood of shifting preferences for chemical prey cues through experience. There were also some sex effects in the final test regarding overall preferences for prey stimuli.

CHAPTER 5

SITE CHOICE

Introduction

Animals are constantly confronted by a great deal of sensory information regarding their environment. Some information can be utilized directly, such as cues left by potential prey which may mark a good foraging site, while conspecifics also leave information that may be of use.

Several studies have demonstrated that some species of garter (Thamnophis) and water (Nerodia) snakes prefer substrate soiled by conspecifics (Allen, Burghardt, and York, 1984; Porter and Czaplicki, 1974; Scudder, Stewart, and Smith, 1980). Dundee and Miller (1968) were among the first to postulate that feces may be an attractant in snake aggregations. There are several reasons why snakes may be attracted to conspecific feces, including gleaning information regarding the resources of an area or the characteristics of the conspecifics leaving the cues.

Feces produced by snakes on differing diets may provide information regarding local prey to other snakes, may then be used in choosing foraging areas, and perhaps influence aggregative behavior. Many studies have demonstrated the ability of snakes to follow chemical

trails of prey items (Chiszar and Radcliffe, 1976; Golan et al., 1982; Halpern and Kubie, 1983; Kubie, 1977; Kubie and Halpern, 1978; Noble and Clausen, 1936), and plains garter snakes, T. radix, have been shown to choose sides of a test arena marked with surface cues of prey species corresponding to the preferences shown in Chemical Prey Preference testing (Burghardt, 1990).

Ward and Zahavi (1973) proposed the "information center" theory in which it was noted that roosting birds somehow shared information regarding the location of distant foraging sites. This phenomenon has been extended into the notion of information transfer (or extraction), and can be accomplished by chemical cues that can be completely passive on the part of the original information "possessor" (Galef, 1986a; Porter, McFadyen-Ketchum and King, 1989). In fact, red-backed salamanders, Plethodon cinereus, have been shown to prefer sites marked with feces of conspecifics feeding on a preferred diet (Walls, et al., 1989). It is possible that such a transfer of information may be occurring via conspecific feces in snakes.

Information afforded by feces is not limited to what an animal has been eating, however. Plethodon cinereus also use pheromonal cues in conspecific fecal pellets to assess competitors for territories, and adjust their behavior accordingly (Jaeger, 1981, 1986; Mathis, 1990).

Alternatively, hatchling green iguanas, Iguana iguana, appear to utilize feces in identifying siblings for formation of aggregative groups (Werner et al., 1987).

A study with plains garter snakes, T. radix, demonstrated that this species can distinguish between feces of conspecifics raised on different diets and will show site preferences based on these feces cues (Burghardt, 1990; Burghardt and Lyman-Henley, in preparation). Thamnophis radix preferred sites marked with prey that corresponded with chemical extract tests of prey preference. However, the same snakes showed a preference for sites marked with feces based on the unfamiliar prey. This was especially pronounced in animals fed solely on earthworms that preferred sites marked with fish-based feces. Information transfer via feces requires that the animals be able to discriminate among feces of animals feeding on different prey, which is what the latter study has demonstrated, but generally is interpreted as involving choices related to acquisition of food. Therefore, the choice of sites marked with feces based on the unfamiliar prey seems counterintuitive, especially when sites marked with the surface cues of the familiar prey were chosen in the corresponding tests.

Thamnophis butleri, a dietary worm specialist closely related to T. radix, also discriminated between feces based on different prey, in a similar experiment

comprising this author's Master's thesis (Lyman, 1990). These snakes also demonstrated a preference for the feces containing unfamiliar prey cues. Thamnophis butleri showed an initial preference for worm-based feces. After experience, those fed worms shifted preference to fish based feces, while those fed fish retained an affinity for worm based feces. Two closely related species demonstrating the same direction in affinity for conspecific feces cues based on diet demonstrated that this is a phenomenon requiring further investigation as to possible functions. It may be that information transfer functions in this case to reduce competition with other snakes feeding on the same prey rather than to locate food itself. This is supported by the fact that T. butleri, unlike T. radix, did not show preferences for sites based on prey cues alone regardless of chemical prey preferences.

In Chapter 4 the chemical prey preference of T. sirtalis subjects were reported. Whereas these were generally in favor of familiar prey, responses to both natural prey remained high regardless of dietary experience. In the following experiment, T. sirtalis raised on specific diets were tested for preferences between sites marked with surface chemical cues of the prey items. Subjects were also tested for site preference based on feces from conspecifics on the same or different

diets. The responses of these experiments were compared to each other and to those based on feces prior to any feeding experience.

Based on previous work regarding site choice of garter snakes relative to prey and conspecific cues, the following hypotheses are proposed:

1. Thamnophis sirtalis will prefer sites marked with chemical cues corresponding with preferred (generally familiar) prey. The similarity in feeding preferences of T. sirtalis with previously tested T. radix should result in a similar use of environmental cues regarding location of preferred prey.

2. Thamnophis sirtalis will prefer sites marked with feces of same-sex conspecifics fed an unfamiliar diet. This has been the case in both species tested thus far, and it is likely that the same mechanisms, such as reduction of competition, may underlie this behavior in the current subjects.

Methods

Subjects

Subjects included a total of 61 T. sirtalis. Their history and maintenance is detailed in Chapter 2. Fifty-nine snakes were utilized in each test, 55 of which were continuous between all tests. Two animals included in the

initial test (both litter 10 females assigned to a worm diet) died before group housing situations were finalized (see Chapter 2). Two other snakes excluded from the Initial Feces-Based Choice Test (16AC due to small size, 10AC due to a slight spinal kink) were therefore reintroduced for later testing, since their responses to the Initial Chemical Prey Preference tests indicated that these animals were responsive and healthy.

Subjects were assigned to diets of nightcrawler or mosquito fish such that each regime was balanced as well as possible for sex, litter, size, and Initial Chemical Prey Preference (described above, Chapter 4).

General Site Choice Testing Procedure

All Site Choice testing was performed in visually isolated individual plastic containers with paper substrate and "tent" shelters (Lyman, 1990). Shelters were positioned on both sides of a central water dish and each covered a 3 x 3 cm stimulus square. One of these stimulus squares bore fish cues, the other cues from earthworms, preparation of which depended on the specific test (detailed below). Stimuli were either surface extracts of fish or worm, or feces from conspecifics feeding on one of the two prey. These squares were fixed to the substrate 30 minutes before snakes were introduced. Subjects were placed in testing containers at about 1630

hrs. of the day before observations began. Figure 5.1 depicts this arrangement. Positions of the two stimuli were alternated, balancing for group, litter, sex and diet. Test boxes were rotated 180 degrees halfway through the series of observations.

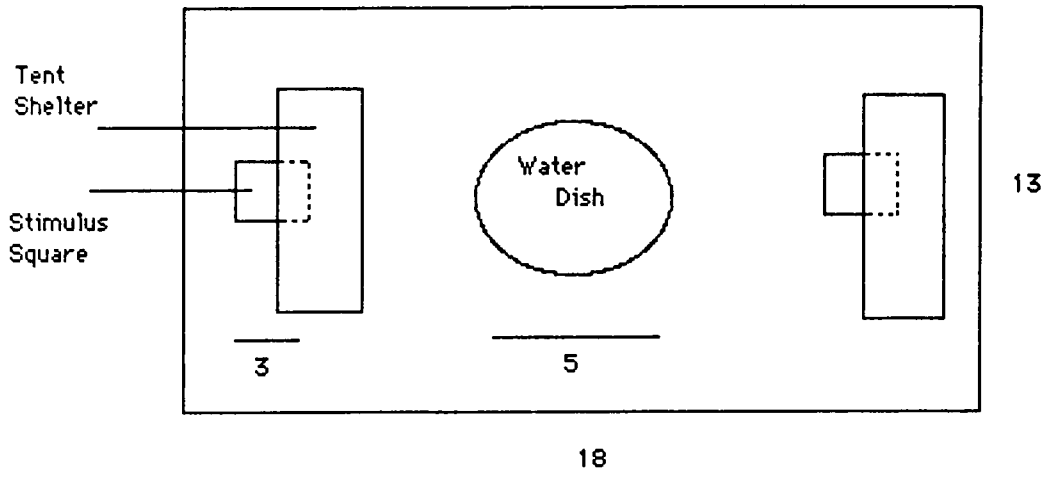
Each observation consisted of noting the placement of each snake as being on the side of the test container associated with worm cues, fish cues, or in the center. Animals were then gently moved to the center of the container in order to encourage choosing again.

A total of three site-choice tests were performed: Prior to feeding experience, a test of initial Feces-based site choice was performed. After experience, two more site choice tests were performed, one using chemical prey cues, and the other conspecific feces (as in the initial test). An initial test of site choice based on chemical prey cues was not performed due to time constraints, since animals had only a limited amount of time for testing before requiring food.

Feces-Based Choice: Initial Test

Thamnophis sirtalis were tested for initial feces-based site choice at 12 days of age. Testing was performed in individual 13 x 18 x 5 cm containers as shown in Figure 5.1A. These containers were the same size as the subjects' home tanks. The stimulus squares were fixed

A)



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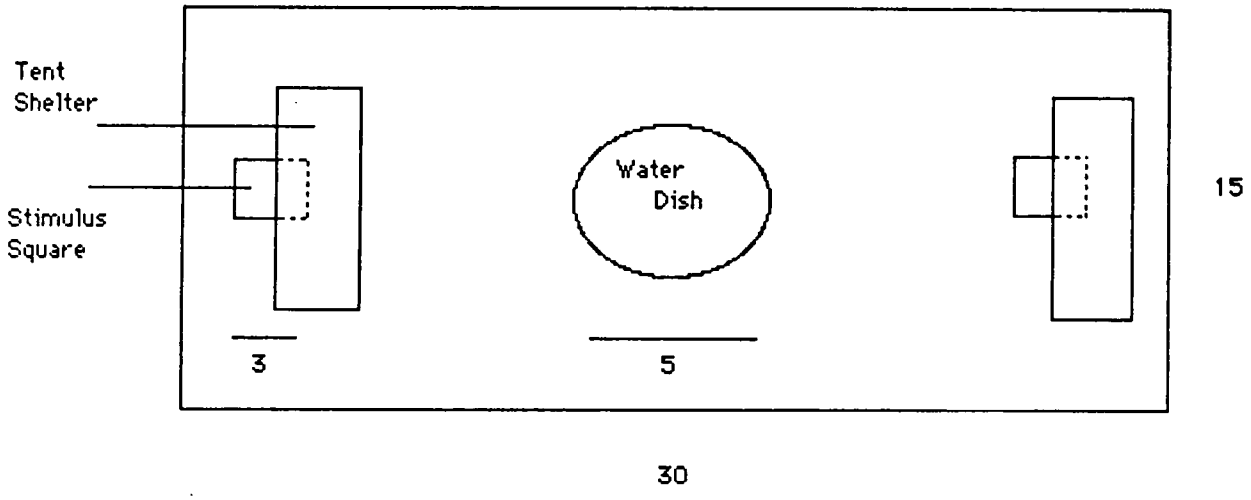

Figure 5.1: Thamnophis sirtalis Site Choice test boxes for A) the Initial Feces-Based Choice Test and B) the Prey Surface-Chemical Choice Test and the Final Feces-Based Choice Test. Distances are shown in cm.

to the cardstock substrate with a small amount of Elmer's© white glue.

In this test, stimulus squares bore feces from older, unfamiliar same-sex conspecifics from the same wild populations. Donors were fasted for 12 days, then fed only Gambusia or Lumbricus on each of at least four days before collection of feces was begun. Feces were collected several times daily and smeared with a clean wooden spatula directly onto the stimulus squares, in this case 3 cm square cardstock. These squares were then frozen until use.

Observations were made three times daily (at 0900, 1200, and 1500 hrs) for two days. The temperature in the testing room was 27°C, the humidity 45±1%. The 12-hour light cycle began at 0600 hrs.

In this test, containers were positioned with the stimuli perpendicular to the experimenter's line of view, such that one was "to the left" and the other "to the right," and containers opened toward the front. There were no discernable light or heat gradients in the room.

Prey Surface-Chemical Choice Test

Thamnophis sirtalis began Prey Surface-Chemical Choice tests at 121 days of age. Containers used in this test were of 15 x 30 x 9 cm clear plastic as illustrated in Figure 5.1B. At this time, animals were maintained in

group housing situations in tanks of this size (detailed in Chapter 2).

Stimulus squares were cut from filter paper and fixed to butcher-paper substrate with double-sided cloth tape. Elmer's© glue was not used (as in the Initial Feces-Based Choice test) due to the liquid nature of the extract, which may have dissolved the water-based white glue. This may have changed the odor of the adhesive, or allowed displacement of stimulus squares during testing, neither of which were acceptable outcomes. One drop of Gambusia or Lumbricus chemical surface extract (such as that used in Chemical Preference Tests, Chapter 4) was placed on each stimulus square and allowed to dry before introduction of test animals. Application was repeated daily after the reading taken at 0900 hrs.

Observations were made three times daily (again at 0900, 1200, and 1500 hrs) for four days, totaling 12 readings. Animals had not eaten for four days prior to testing, which was the standard between-meal period.

This test was conducted in a walk-in Nor-Lake, Inc. environment chamber with a 11:13 light:dark cycle beginning at 0700 hrs. The temperature was 24°C during the light period and 21°C when dark. Humidity was 39±8%.

In the environment chamber it was necessary to position testing containers on the shelves such that one stimulus was toward the wall and the other closer to the

experimenter ("back" and "front"). This was perpendicular to the position direction used in the initial test. This positioning resulted in a slightly decreasing light gradient toward the "back" of the container, as well as that side being consistently farther away from the experimenter during observations.

Feces-Based Choice: Final Test

Thamnophis sirtalis began the final test for feces-based site choice at 135 days of age. Procedures were identical to those for the Prey Surface-Chemical Choice Test described above except for the stimuli. Stimulus squares were cardstock bearing feces obtained in the same manner as in the Initial Feces-Based Habitat Choice test described above, fixed in place with double-sided cloth tape. Position of fish and worm cues were reversed in relation to the Prey Surface-Chemical Choice Test. The daylight temperature was 23.5°C and humidity 45±6%.

Data Analysis

Choice scores were obtained by subtracting the number of site choices in favor of worm from those for fish. Animals with negative choice scores were subsequently counted as preferring the site marked with worm cues, those with positive scores were recorded as preferring fish-based cues. Chi-Square tests were performed using

the numbers of individuals showing preferences in favor of each stimuli. Changes in preference between initial and final feces-based choice tests were analyzed using the McNemar test for the significance of changes (Siegel and Castellan, 1988).

Responses that resulted in "ties" were eliminated from statistical analyses. These included observations in which the animal was located in the center of the tank (Initial Feces-Based Choice Test occurrences: 2 of 354 observations; Final Feces-Based Choice Test: 7 occurrences of 708 observations; Prey Surface-Chemical Choice Test: 4 occurrences of 708 observations). Instances in which different Choice scores summed to zero were also eliminated as ties from Chi-square analysis as being non-preferential (Initial Feces-Based choice test: 17 of 59 occurrences, Final Feces-Based Choice Test: 13 of 59 occurrences, and Prey Surface-Chemical Choice Test: 9 of 59 occurrences).

Analyses were performed to compare the responses of animals of different litters, sexes, diets, and housing groups among the experiments here performed. Housing groups were analyzed both using all groups, and with responses pooled between like groups (Fish-fed groups 1 and 2, worm-fed groups 3 and 4, mixed diet groups 5 and 6). Responses to position of the container in the room (side rather than stimulus preference) were also subjected

to Chi-square tests. All Chi-square contingency tests were analyzed using Statgraphics 4.0 (STSC, 1990). The McNemar tests were calculated as per Siegel and Castellan (1988) using a hand calculator, as were all other chi-square tests.

Results

Initial Feces-Based Choice Test

Overall, animals' responses were significantly biased in favor of worm-based feces (Chi-square=5.76, df=1, $p<0.02$), frequencies of choices being 27 of the individuals in favor of worm, 12 in favor of fish, and 20 with no difference. Preferences for worm-based feces were significant in litter 10 snakes (Chi-square=5.56, df=1, $p<0.02$), males (Chi-square=4.26, df=1, $p<0.05$), and animals later assigned to a fish diet (Chi-square=4.17, df=1, $p<0.05$). Chi-square contingency tables found no significant effects of litter, sex, or assigned future diet or group on the responses to diet-based feces in this test. Table 5.1 summarizes these results for this initial test.

There was a nonsignificant bias in favor of the left side of the tank, with 27 animals more often on the left and 17 more often on the right. After rotation of test boxes, 23 of the animals spent more time on the opposite

Table 5.1: Number of T. sirtalis prior to feeding experience showing preferences for sites marked with feces of conspecifics on different diets.

Variable		N	Preference: Fish	Preference: Worm	Preference: None	Chi-square Contingency Tests
Future Diet:	Fish	30	7	17*	6	n.s
	Worm	29	5	10	14	
Litter:	10	29	4	14**	11	n.s
	16	30	8	13	9	
Sex:	Female	32	7	13	7	n.s
	Male	27	5	14*	13	
Total:		59	12	27*	20	

Note: "*" denotes chi-square $p<0.05$,
"**" denotes chi-square $p<0.02$.

stimulus side from the first three observations. In other words, they tended to remain on the same side of the container relative to the testing room, although the stimulus squares had changed position. However, only 6 of these animals showed a stronger affinity for side than stimulus, and never by more than 2 points. Such individuals wee not excluded from analyses since observable preferences for stimuli were still present.

Prey Surface-Chemical Choice Test

There was a significant preference for sites marked with worm prey surface cues (chi-square=9.0, df=1, $p<0.01$), with 35 of the snakes choosing worm cues, 14 fish cues, and 10 showing no preference. These results are seen in Table 5.2. Within diets, animals fed worms showed a significant preference for the familiar prey surface cue (chi-square=8.91, df=1, $p<0.01$), but fish-fed animals showed no significant preference. Similarly, worm cues were significantly preferred by litter 10 snakes (Chi-square=6.0, df=1, $p<0.02$) and males (Chi-square=6.55, df=1, $p<0.02$). Contingency tests found no significant effects of sex, litter or group on choices based on Prey Surface.

Regarding tank position, 37 of the animals were significantly more often found in the back half of the box regardless of stimulus position (chi-square=17.0, df=1,

Table 5.2: Numbers of T. sirtalis after feeding experience showing preferences for sites marked with chemical surface extract of prey items.

Variable		N	Preference: Fish	Worm	None	Chi-square Contingency Tests
Diet:	Fish	30	10	17	3	n.s.
	Worm	29	4	18**	7	
Litter:	10	28	6	18*	4	n.s
	16	31	8	17	6	
Sex:	Female	31	9	18	5	n.s.
	Male	28	5	17*	5	
Total:		59	14	35*	10	

Note: "*" denotes chi-square $p<0.02$,
"**" denotes chi-square $p<0.01$.

p<0.001), only 9 being found in the front and 13 with no difference. Twelve animals showed stronger preferences based on side than stimulus position. Again, such animals were not excluded from analyses since they still exhibited measurable preferences based on prey stimuli.

Final Feces-Based Choice Test

There was a significant effect of sex (Contingency chi-square=12.6, df=1, p=0.0004) in that females preferred fish cues while males preferred worm. Within the sexes, males significantly preferred worm cues (chi-square=12.8, df=1, p<0.001), though female preference for fish cues did not reach significance. Frequencies of choices for fish or worm based feces cues were not significantly different, but still showed a slight bias toward worm cues (27) over fish (17). Worm-fed snakes showed this bias more clearly (15 vs. 7, respectively) than fish-fed snakes (12 vs. 10). There were no significant effects of litter or housing group. Table 5.3 shows these results.

With respect to which sides of the container were frequented regardless of stimulus position, 38 of the snakes were found significantly more often in the rear of the tank (Chi-square=14.88, df=1,p<0.001), 11 in the front, and 10 showed no preference. Eight animals were more strongly associated with side than stimulus position.

Table 5.3: Number of *T. sirtalis* after feeding experience showing preferences for sites marked with feces from conspecifics on different diets.

Variable		N	Preference			Chi-square Contingency Test
			Fish	Worm	None	
Diet:	Fish	30	10	12	8	n.s.
	Worm	29	7	15	7	
Litter:	10	28	6	14	8	n.s
	16	31	11	13	7	
Sex:	Female	31	15	9	4	X =12.6 p=0.0004
	Male	28	2	18*	11	
Total:		59	17	27	15	

Note: "*" denotes chi-square p<0.0001.

Change Between Feces-Based Tests

McNemar tests of change did not find any significant changes of preference between the initial and final feces-based choice test, since 8 animals changed from fish to worm preference and 10 from worm to fish. In fish-fed animals the change was most pronounced, with 4 changes from fish to worm but 8 from worm to fish. Worm-fed animals similarly only showed 2 changes from worm to fish and 4 from fish to worm. Note that there did tend to be more changes in favor of the familiar feces than against it. Figure 5.2 illustrates this. There were no significant changes between tests relative to sex or housing group.

Figures 5.2 and 5.3 are representations comparing F-W score responses of individual snakes between two tests. Figure 5.2 presents information regarding the F-W Choice scores of animals in both the initial and final Feces-Based Choice Tests, in which positive scores reflect choices in favor of fish cues, negative scores in favor of worm cues, the distance from zero showing the strength of the preference. Figure 5.3 compares the Prey Surface-Chemical and Final Feces-Based Habitat Choice Tests.

There was a significant positive relationship between site choices based on Prey Surface cues and those based on Feces-Based cues in the final test (Chi-square=8.74, df=1,p=0.0038). This is illustrated by Figure 5.3. The

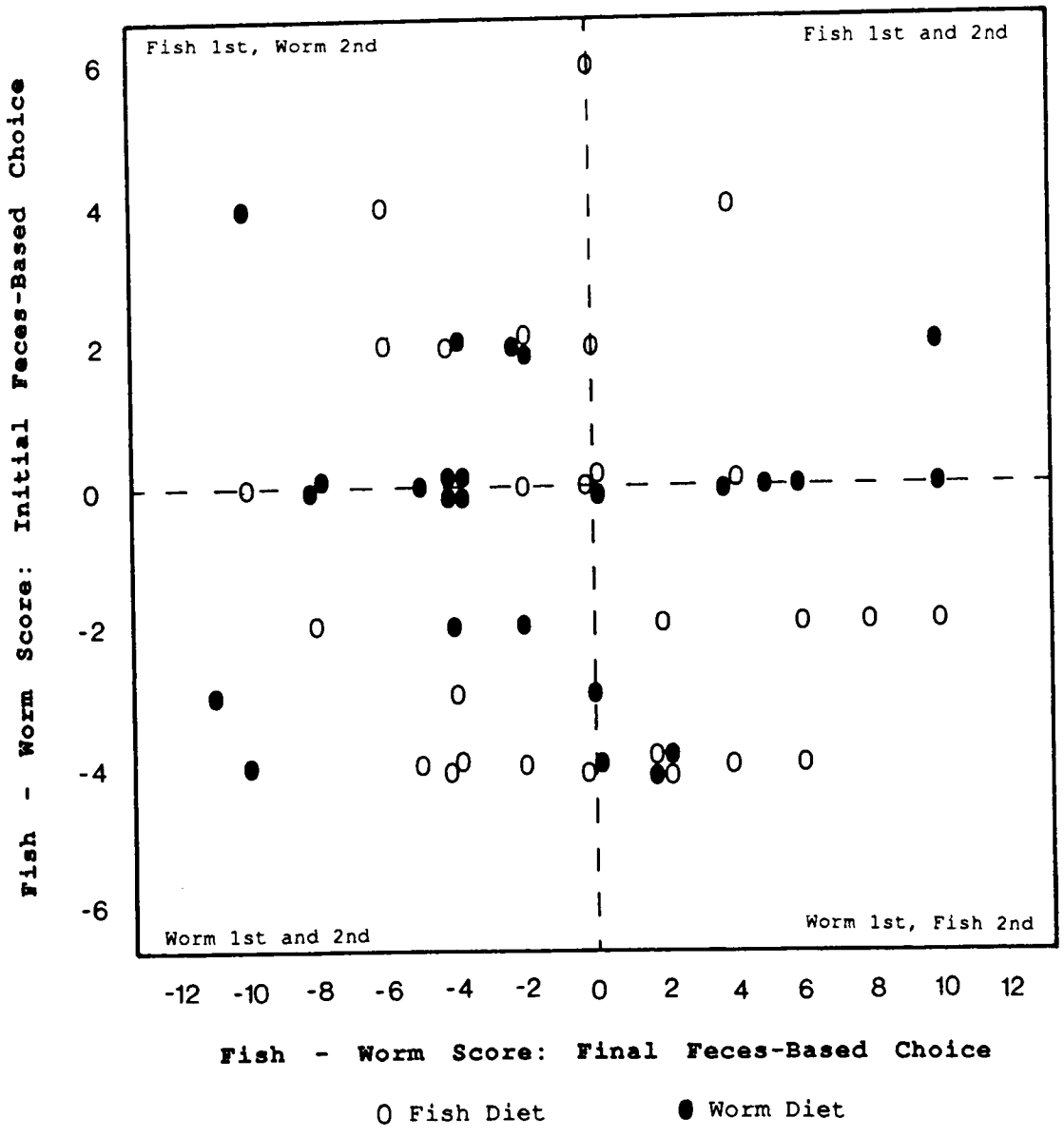

Figure 5.2: Relationship between Feces-Based Site Choices in Thamnophis sirtalis on different diets before and after dietary experience.

Note: Responses toward worm cues are subtracted from those toward fish cues to produce a score such that negative values reflect preferences for worm cues, positive values for fish. Stronger preferences are reflected by the difference from 0.

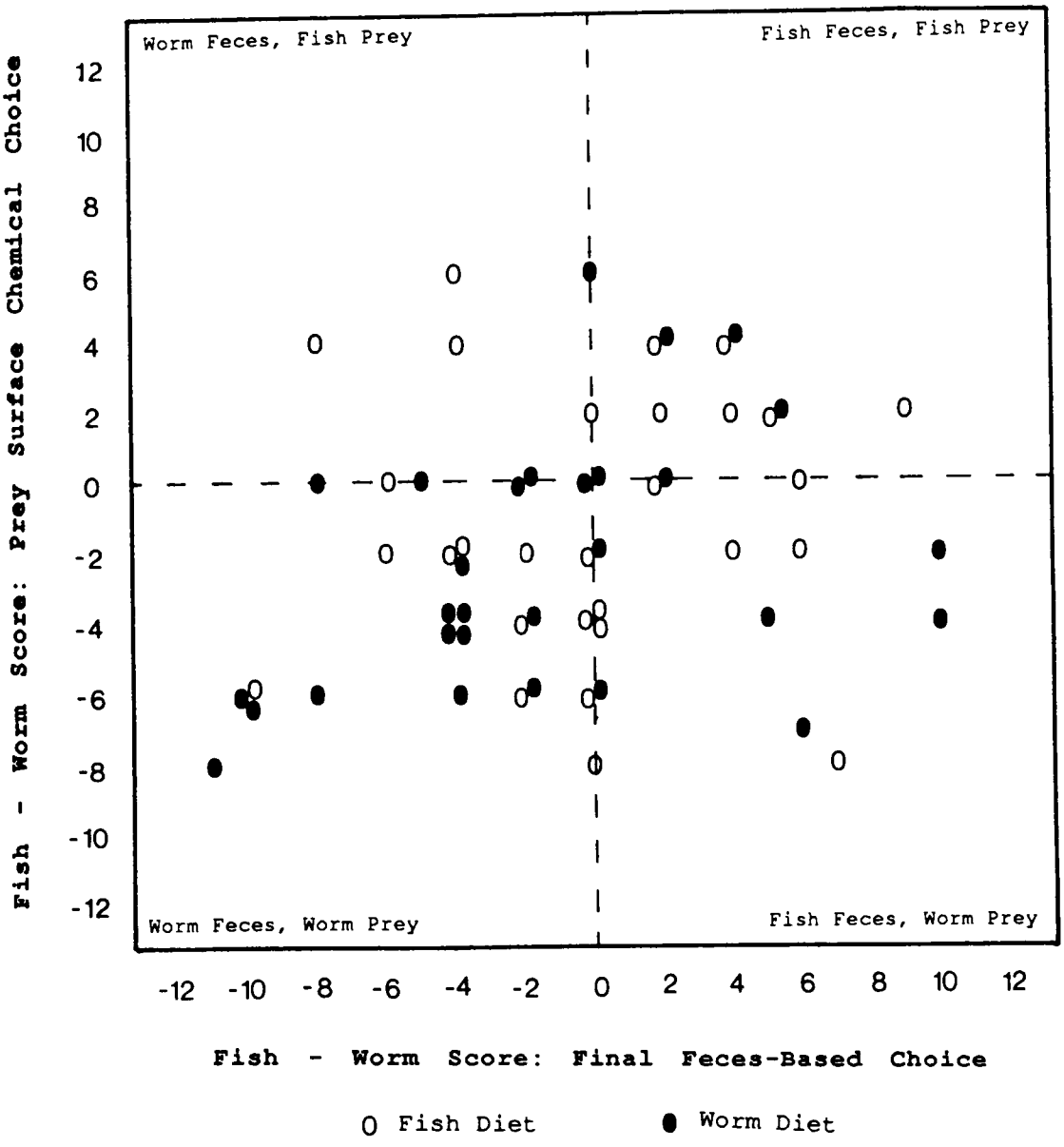

Figure 5.3: Effects of diet on the relationship among Prey Surface-Extract and Feces-Based Site Choices in Thamnophis sirtalis on different diets.

Note: Axes are as in Figure 5.2.

least likely combination of responses was a preference for fish prey cues and worm feces cues (3 occurrences). A combination of preferences for both worm prey cues and worm feces cues was much more common (19 occurrences) than the fish/fish (9 occurrences) and worm prey/fish feces (7 occurrences) combinations.

Discussion

The role of prey cues in site choice for T. sirtalis shows a direct relationship to the dietary regime of these animals. Prior to any experience with feeding (or defecation), these subjects showed a significant bias in favor of feces carrying worm cues. This corresponds with the Initial chemical prey preference (Chapter 4) which was also biased toward worm for these animals.

The site choice test based on prey surface cues corresponded with chemical preferences after feeding experience as well. The overall preference was for the sites marked with worm cues. This was strongest in the worm fed animals and weaker in the fish fed ones, the latter animals perhaps reflecting a shift in favor of fish, or at least less interest in worm.

The final feces choice test again shows a general (though non-significant) trend for animals to prefer sites based on feces of conspecifics fed worms. What changes

occurred between tests were most often in favor of familiar prey cues.

The influence of dietary experience on the choice of sites marked with feces (however slight) allows the occurrence of information transfer in which animals would be able to choose sites (foraging areas, etc.) based on conspecific feces carrying preferred prey cues. Both of the previously described studies utilizing T. radix and T. butleri showed contrary results relative to final feces site choice, such that animals preferred feces cues of the unfamiliar diet. The profile of generalist T. radix was otherwise similar to T. sirtalis, with chemical preferences and site choice based on prey cues both in favor of the familiar prey item. It was assumed that these similarities would continue in site choice based on conspecific feces, since the two species have very similar dietary preferences. The worm specialist T. butleri also showed an initial preference for worm-based feces, but showed no site choice based on prey cues, and the distinctive opposite-prey cue choice in final feces choice testing that had been seen in T. radix. The results with T. butleri had suggested a function other than site choice based on prey availability, possibly based on familiarity instead. It was postulated that animals may use novel feces cues as a mark of an unfamiliar animal rather than information regarding food, and familiar conspecifics may

then be avoided, perhaps to reduce competition or aid in dispersal.

Thamnophis sirtalis shows an overall pattern more consistant with the original hypothesis of choice based on prey items, or at least on familiarity rather than unfamiliarity. The significant effect of sex on feces based site choice may be related to the phenomenon seen in T. radix and T. butleri. Thamnophis sirtalis females preferred sites marked with fish based feces cues, whereas males showed a preference for worm based cues. The sex effect seen regarding chemical prey preference (Chapter 4) is in the opposite direction, with females preferring worm prey cues and males preferring fish. Is this pattern due to a similar underlying function to the phenomenon as seen in T. radix and T. butleri?

Thamnophis sirtalis seemed to show only a relatively weak interest in the test stimuli, many individuals simply preferring the back of the cage regardless of stimulus cues offered. Unfortunately, this limited the number of snakes showing preferences based on stimuli, making interpretation of the results that much more difficult. It is possible that the cues offered were not as relevant to these subjects as they were to the other species used. Alternatively, external environmental conditions may have been more important, such as the difference in light levels between sides of the test containers. Similarly,

disturbance from the investigator always came from the "front," and it may be that some snakes were more interested in avoiding this disturbance than in attending to the test stimuli. It has been found that T. sirtalis tend to be more reactive in defensive behavior than either T. radix or T. butleri, and this may have effected the cooperation of the subjects in this study (Herzog, Bowers and Burghardt, 1989).

In conclusion, T. sirtalis showed an initial preference for sites marked with feces of conspecifics fed earthworms. After experience with prey, sites were chosen based on prey surface cues of the familiar diet with an overall bias toward worm. Choices based on conspecific feces cues tended to match the choices that had been made regarding prey surface cues, such that animals overall preferred sites marked with worm-based feces, with animals tending to choose feces based on familiar prey. There was an effect of sex regarding this final feces choice test which was in the opposite direction to that seen regarding chemical prey preference, such that males preferred worm-based feces cues although preferring fish chemical prey cues (Chapter 4).

CHAPTER 6

AGGREGATION

Introduction

Although most snakes are "low density carnivores rarely found in groups" (Burghardt, 1983, p. 99), several species are known to aggregate. Well-documented cases of snake aggregation occur in conjunction with reproductive activity (e.g., Crews and Garstka, 1982) and hibernation (e.g., Duvall, King, and Gutzwiller, 1985). However, snake aggregations occur at other times as well, and may serve a wide variety of functions. Thermal stability, and reduced oxygen consumption or water loss have been demonstrated in some snake aggregations (Aleksiuk, 1977; Noble and Clausen, 1936; Myers and Eells, 1968). Protection from predation (Gregory, 1975), enhanced foraging (Barbour, 1962; Kropach, 1971), and increased growth rate (Burghardt, 1990) have also been suggested.

It has also been postulated that snakes in aggregations could be engaging in information transfer (or extraction) wherein animals may obtain information regarding the diets of other snakes in an aggregation Burghardt, 1990; Lyman, 1990). An influence of diet on association pattern has been found in spiny mice, Acomys

caharinus, that prefer animals fed upon a familiar flavored diet (Porter, McFadyen-Ketchum, and King, 1989).

Several additional influences on aggregation have been suggested in other species including kinship, familiarity, and sex. The theory of kin selection refers to increasing an animal's inclusive fitness by helping near relatives (e.g., Hamilton, 1964). Preferentially aggregating with siblings may serve this function by sharing the various benefits of group living with close kin. This phenomenon has been demonstrated in several species of anuran tadpoles (e.g., Blaustein and O'Hara, 1983; Waldman, 1985). Similar examples of kin recognition and preferential association are found in Japanese quail chicks (Waldman and Bateson, 1989), female Belding's ground squirrels (Holmes, 1986a), and hatchling green iguanas (Werner, et al., 1987).

For many animals, familiarity virtually guarantees kinship, so further mechanisms of kin recognition are unnecessary. Voles and deer mice have been shown to prefer animals that they had been reared with, regardless of genetic relatedness (Dewsbury, 1988a, 1988b; Ferkin, 1990; Halpin and Hoffman, 1987). Further, upon sexual maturity animals often begin showing preferences to associate with the opposite sex (Drickamer, 1989; Hurst, 1990; Lyman-Henley, personal observation).

In a previous investigation into snake aggregation behavior, Lyman (1990) observed 24 Butler's garter snakes, Thamnophis butleri, in a large arena with 8 identical shelters. This study included juvenile animals controlled for diet and familiarity and balanced by litter and sex. Snakes were found to cluster non-randomly under shelters such that animals tended to be most closely associated with unfamiliar littermates (siblings separated within 24 hours of birth) fed different diets.

These results seem to indicate an interaction of various mechanisms underlying aggregation in T. butleri. A preference for siblings corresponds to the work mentioned above in tadpoles, iguanas, and other species. However, the preference for unfamiliar animals appears here to be unrelated to kin recognition. Yeager and Burghardt (in press) found that T. radix preferentially associated with conspecifics that they had not competed with over food. It may be that T. butleri associated familiarity with competition, although the subjects never actually interacted over food. Animals that have encountered each other in the same discrete area may be assumed to more likely have been competitors for resources than animals that have never met before. Therefore, T. butleri showing a preference to associate with unfamiliar animals may have been avoiding "competitors." The preference for animals on an unfamiliar diet may have

similarly shown an avoidance of animals feeding upon preferred prey. Unfortunately, familiarity and diet were confounded in that study due to small numbers of subjects.

To further investigate the influence of diet, kinship, sex, and familiarity on the aggregative behavior of snakes, the grouping patterns of 38 T. sirtalis were observed in a large arena with several identical shelters. A greater number of subjects allow replications of housing groups, eliminating the confound of diet and familiarity presented in Lyman (1990). The subjects were similarly derived from 2 litters, and differed in diet and interaction histories as detailed in Chapter 2. In previous chapters a preference for chemical cues of familiar prey has been demonstrated in these test animals, as has a tendency to choose the side of a container marked with feces from an animal feeding on familiar prey.

In Chapter 1, the following hypothesis was stated:

"5. Thamnophis sirtalis will aggregate preferentiallly with unfamiliar littermates feeding on the unfamiliar diet. There is a lack of information regarding aggregation patterns based on diet, kinship, sex, or familiarity in T. sirtalis. Therefore, assuming that the previous four hypotheses are correct and the underlying mechanisms of the behavior are similar, I tentatively predict that T. sirtalis will parallel T. butleri in aggregation pattern. (ibid: p. 35)."

At this point I will review and adjust this basic prediction into more specific hyptheses based upon the literature and results reported herein.

Thamnophis sirtalis has been described as a snake that forms aggregations outside of hibernation and reproductive periods, and has been used in studies of aggregative behavior (e.g., Burghardt, 1983; Heller and Halpern, 1982a). This leads to the first hypothesis of this chapter:

1. Aggregations are expected to form nonrandomly under physically identical shelters.

The second hypothesis deals with kinship. Based on the earlier work with T. butleri and the literature on other species, I predict that:

2. Thamnophis sirtalis will preferentially associate with siblings.

In Chapter 5, the subjects were shown to have a slight preference for sites marked with feces of conspecifics on familiar, preferred, diets. This is contrary to the findings in T. butleri, which correspond with an association with animals on different diets. Therefore, the third hypothesis of this experiment has been suggested:

3. Thamnophis sirtalis subjects will preferentially associate with animals feeding on the familiar diet.

Lastly, T. butleri had grouped with unfamiliar animals, though this may have been confounded with diet. In light of Yeager and Burghardt's (in press) work, I believe that an attraction to unfamiliar animals is reasonable despite the aforementioned difference in use of feces cues. Therefore, I shall predict that:

4. Unfamiliar individuals will be preferentially associated within aggregations of juvenile T. sirtalis.

Methods

Subjects

Thamnophis sirtalis housed only with members of their own species (groups 1 through 6 and 12 [isolates]) began testing at 218 days of age. Fifteen animals from litter 10 (Worm diet: 4, fish diet: 11) and 23 from litter 16 (worm diet: 11, fish diet: 13) were tested. Table 6.1 summarizes the diet, sex, litter, and housing group of each subject. Maintenance of these animals is detailed in Chapter 2.

The numbers of test animals available for this experiment were decreased from the earlier testing sessions due to subject mortality, resulting in a less-balanced design. It is believed that toxic Lumbricus caused the deaths of 10 of these 12 animals, as was detailed in Chapter 2 (see also Lyman, 1990). All animals

Table 6.1: Composition of groups of Thamnophis s. sirtalis (N=38) used in arena aggregation test.

			SUBJECT	
GROUP	DIET	LITTER	MALE	FEMALE
1	FISH	10	D, K	N, AB
		16	Y, AH	V, AF
2	FISH	10	S, V	Q, AD
		16	J, AE	S, AD
3	WORM	10	X	Z
		16	X	E, K
4	WORM	10	-	-
		16	G, H	C
5	FISH	10	L	M
		16	AB	R
	WORM	10	Y	P
		16	U	AJ
6	FISH	10	W	-
		16	M	T
	WORM	10	-	-
		16	W	-
"12"	FISH	16	-	P
	WORM	16	-	D

NOTE: Group "12" refers to individually housed animals.

used in this aggregation test appeared to be behaving normally and in good health.

Test Apparatus

Tests were conducted in a Nor-Lake, Inc. Environment Chamber with an 11:13 light:dark photic cycle (lights on at 0800 hrs.) and a 9:15 warm:cool thermal cycle (23°C starting at 0900 hrs, 18°C starting at 1800 hrs.). Temperature changes were gradual over the course of about 45 minutes. A centrally located overhead flourescent fixture provided light.

An oblong, galvanized metal trough was used as the testing arena, and is diagrammed in Figure 6.1a. Inside measurements were 63 x 123 cm, and 59 cm in height. The arena was supported on a four inch thick foam rubber pad to absorb vibrations from the environment chamber. Clean aquarium-grade gravel covered the floor of the arena to a depth of 1 1/2 inches. Gravel was rinsed thoroughly with hot water and allowed to dry before use.

Cover objects, shown in Figure 6.1b, were made of clear plexiglass with central upright pegs and three suspending "feet" of plexiglass and wood raising the object off the substrate by about 10mm. Wooden disks with holes for fitting over the plexiglass uprights provided opacity, and could be removed without lifting the plexiglass to allow viewing of snakes under the cover

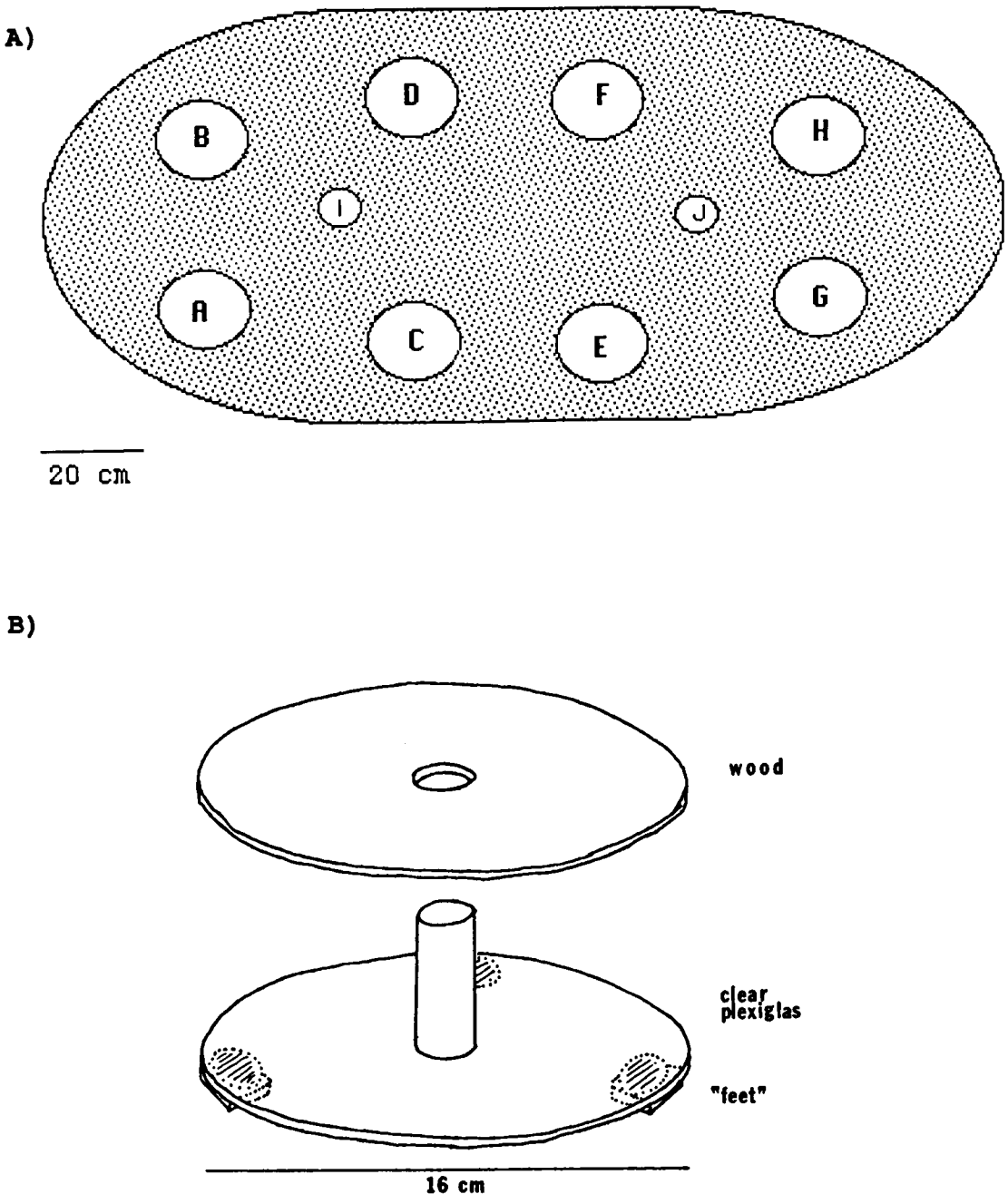

Figure 6.1. A) Diagram of arena used in observations of aggregation in T. sirtalis. Circles labeled A - H represent cover objects (16 cm diameter), circles I and J represent water dishes (10 cm diameter). B) Cover objects used in arena aggregation tests of T. sirtalis.

object. Cover objects were 16 cm in diameter and labelled A through H, corresponding to position within the arena as shown in Figure 6.1a. These covers had been successfully used in previous aggregation experiments (e.g., Lyman, 1990; Schwartz, 1989), and had been modified by the attachment of the wooden portion of the "feet" to make them suitable for the current subjects (see Lyman, 1990). Water dishes were 10 cm diameter plastic petri dishes.

Cover objects were arranged in the arena so that the closest arena wall was 5 cm from the edge of the object, and all objects were 12 cm from their neighbors. The four central cover objects, C through F, were 21 cm from the cover on the opposite wall of the arena. Water dishes were positioned 10 cm from neighboring cover objects. The center of the arena was left without a water dish and was the site where all animals were introduced to the trough.

Test Procedure

After being fed early in the morning, the snakes were introduced to the arena at about 1630 hrs. on the day before observations were to begin. All animals in a test were placed in a metal canister and gently introduced together into the center of the testing arena. The animals were undisturbed except for two position readings taken daily, for four consecutive days, for a total of eight readings. Position readings were taken at 0730 hrs.

and 1830 hrs., when the environment chamber was dark and at 18°C. This was in order to assess the resting positions of the animals unconfounded by patterns of diurnal exploration. Earlier observations made of the subjects in the testing apparatus had suggested that animals emerged from cover at first light and retreated under them again when the temperature began to fall for the evening (Lyman-Henley, personal observation). A total of 304 snake observations (38 snakes by 8 observations) were made.

Each reading consisted of recording the position of all animals in the arena, collecting them, and re-introducing them into the center of the tub. This was achieved by scanning the arena with a partially masked flashlight and recording the positions of the animal's heads. Positions were recorded to scale on a diagram of the testing arena at each reading. Animals not under cover were recorded and collected first, then each cover object was checked, in order from A through H. The wooden disk was removed and the positions of any snakes under that cover quickly recorded, any animals touching being noted. The plexiglass cover was then lifted and animals collected. Snakes would begin to move and leave the cover soon after being exposed to light, so this step had to be made quickly. If any animal left cover during a reading but before its position had been recorded, both the place

it exited the cover and the direction travelled were noted. After all snakes were collected, cover objects set back in place, and any other activities within the chamber completed, animals were re-released into the center of the arena.

Data Analysis

Four main aspects of the aggregation data were analyzed: A) Distribution of Snakes; B) Nearest Neighbor Distance (NND); C) Overall Nearest Neighbor (ONN); and D) Incidence of Shared Shelters (ISS). These are defined as follows.

Distribution of Snakes. This refers to whether grouping behavior was occurring in the arena observations. The frequency of cover utilization versus appearance in the open was compared in the subjects. Differential utilization of the various covers was investigated. This concerned both the number of snakes found under a given shelter at a time, what shelters were used most often, and site fidelity of the subjects.

Nearest Neighbor Distance (NND). The distances between each animal were measured directly from the data sheets for each reading. The scale was 1 unit equivalent to 1 cm, and distances were measured between subjects'

heads to the nearest 0.5 unit. If distances between two neighbors were "tied," either a touching animal, or one slightly closer in exact units would be preferentially used as a "nearest neighbor."

The distances between each possible pairing of animals per reading was then summed to provide a single measure of average distance between all possible combinations of animals for the entire observation period. This allowed the generation of Pair NNDs, that reflect the individual averaging the least distance from each subject over all 8 readings.

One thing to note is that minimum distance per reading here is 1, though it is possible to have smaller distances than this between snakes in reality. Also, even animals in physical contact are not necessarily closest at the head, which is the point used as reference here. Alternatively, snakes not in resting positions, but engaged in exploratory behavior, may inflate their mean distances to other individuals.

Snakes recorded as leaving a shelter were, for purposes of measurement, "positioned" at just under the edge of the shelter at the point of exit.

Overall Nearest Neighbor (ONN). The summed distances between each possible pair of snakes that were produced for Pair NNDs (above) were used to determine for each

subject the closest individual over observations in each of the following 8 categories: 1) member of the same sex; 2) opposite sex; 3) same litter; 4) opposite litter; 5) fed the same diet; 6) opposite diet; 7) a familiar animal (housed together); or 8) an unfamiliar animal (not housed together).

Incidence of Shared Shelters (ISS). The number of observations that each subject was found under the same shelter with each other snake determined its ISS. Thamnophis sirtalis tended to begin moving immediately upon lifting of the wooden cover disk, making it difficult to tell exactly what snakes may have been touching each other before disturbed. Therefore, while in earlier work the incidence of physical contact was also used in this measure (Lyman, 1990), in this experiment it was assumed that any snakes under a given shelter may have been touching.

Statistical tests. Pair NNDs were analyzed by density linkage cluster analysis (Hartigan, 1975). Binomial probabilities were generated for other comparisons, as determined by the number of possible outcomes (a baserate) and the number of chances for each outcome to be observed (trials). This generates an expected distribution and so provides a probability for the observed outcome.

Student's t-tests were performed comparing mean NND's of animals of different diets, litter, and sex. All analyses were performed using SAS (SAS, 1985).

Results

Distribution of Snakes

Aggregation. As compared to a binomial distribution, on only 3 of the 8 observations were subjects significantly clumped under shelters (shelters as baserate 1/8 (0.125), snakes as trials 38, $p<0.05$). This is detailed in Table 6.2.

Cover Utilization. While covered areas constituted less than half the area of the arena, snakes were found to be under the shelters in 74%, and out in the open only 26%, of the observations. This is a highly significant difference (8 shelters by 8 readings as baserate 1/64 (0.156), 304 total observations as trials, $p<0.00001$). Table 6.2 includes this information.

Nine individuals were always found under covers, 7 of which were female (Chi-square=2.78, df=1, $p<0.10$). There was no preponderance of one litter (4 litter 10 animals, 5 litter 16) or diet (5 worm fed, 4 fish fed).

There was no difference between the litters regarding overall cover use, with 73% of litter 10 and 78% of litter

Table 6.2: Number of T. sirtalis under each cover or in the open for the 8 aggregation readings, and the mean number of snakes per shelter.

		Shelter								
Rdg.	(out)	A	B	C	D	E	F	G	H	prob.
1	(8)	2	3	7	2	1	3	3	9	p=0.016
2	(17)	3	2	2	1	1	1	3	8	p=0.05
3	(10)	4	5	4	6	3	0	4	2	n.s.
4	(8)	5	2	2	6	3	2	5	5	n.s.
5	(8)	6	3	2	5	1	5	5	3	n.s.
6	(8)	4	11	3	2	1	3	4	2	p=0.0017
7	(10)	4	3	2	5	1	3	4	6	n.s.
8	(9)	5	4	4	1	0	2	6	7	n.s.
Total:	(78)	33	33	21	33	11	19	34	42	
Mean (X=3.5):		4.1	4.1	2.6	4.1	1.4	2.4	4.3	5.3	

Note: "*" illustrate significant differences, "prob." reports the level of significance; "n.s." reports a p-value > 0.1. Refer to Figure 6.1 for positioning of shelters A - H in the arena.

16 snakes being found in the open at least once. However, 47% of litter 10 snakes were out 3 or more times, whereas only 22% of litter 16 snakes were out this frequently.

Thamnophis sirtalis were occasionally found in the open curled in resting positions, in one case 2 animals curled together. Of the 78 observations made of snakes in the open, litter 10 animals were found in resting positions in 22 out of 34 observations (65%), litter 16 animals only curling in the open 11 out of 43 observations (26%). Snakes observed in the open that were actively moving about often came into brief physical contact with each other, but such incidental contact was not scored.

Site Fidelity. Thamnophis sirtalis showed significant site fidelity. Snakes needed to utilize the same shelter on only 2 or more readings to show a significant site fidelity (8 shelters by 8 readings as baserate 1/64 (0.0156), 38 snakes as trials, $p<0.02$). Thirty-one of the 38 snakes (82%) used at least one shélter on 2 or more of the 8 readings. Several snakes showed site fidelity to more than one shelter, 14 using 2 shelters significantly, and 6 using 3 shelters. Among litter 10 snakes, 73% animals showed site fidelity, mostly utilizing only 1 shelter at a significant rate (55%), whereas 27% showed no fidelity to shelters. More snakes in litter 16 showed site fidelity (87%), with most animals

found under 2 different shelters at a significant rate (60%), only 13% showing no site fidelity at all.

Animals were found under an average of 4.1 different shelters over the course of the 8 observations. Snakes did show some favoritism between shelters, 5 inividuals demonstrating their strongest site fidelity to shelter "H," while shelter E was never used by any animal more than once.

Nearest Neighbor Distances (NND)

Mean NNDs for each snake over the 8 readings ranged from 2.6 cm to 11.1 cm, averaging 5.6 cm. This information is included in Table 6.3. Means were slightly lower for females (mean NND= 5.2 cm) than males (mean NND= 5.9 cm). Animals on the worm diet (mean NND= 5.4 cm) and litter 16 (mean NND= 5.3 cm) also averaged slightly smaller separation distances than fish fed animals (mean NND= 5.7 cm) and litter 10 (mean NND= 6.0 cm). Student's t-tests found no significant differences between any of these means.

Pair NND. Another way to consider NNDs involves the smallest mean distances between each possible pair of snakes over the 8 readings (Pair NND). This ranged from a minimum of 25.0 cm to a maximum of 40.5 cm. The mean for all combinations was 32.5 cm. Mean Pair NNDs were

Table 6.3: Description of T. sirtalis subjects and summary of measures of association: NND (nearest neighbor distance), Pair NND (least distance between individuals over 8 readings). All distances are in cm.

Lit	Sub	Sex	Diet	Group	NND mean	NND SE	Pair NND mean	Pair NND Indiv.
10	D	Male	Fish	1	6.8	3.7	38.5	16V
	K	Male	Fish	1	11.0	7.4	40.5	16AB
	L	Male	Fish	5	5.9	3.2	33.6	16S
	M	Female	Fish	5	7.9	2.9	32.0	16AJ
	N	Female	Fish	1	7.8	5.4	33.8	16H
	P	Female	Worm	5	4.5	1.5	33.9	16AJ
	Q	Female	Fish	2	5.1	3.1	31.1	16AD
	S	Male	Fish	2	5.0	2.6	36.8	16W
	V	Male	Fish	2	7.3	2.6	33.4	16AB
	W	Male	Fish	6	6.4	4.7	36.1	16T
	X	Male	Worm	3	4.4	1.9	35.4	10N
	Y	Male	Worm	5	3.8	1.5	28.3	16M
	Z	Female	Worm	3	3.6	1.8	27.3	16G
	AB	Female	Fish	1	5.9	3.9	32.1	10Q
	AD	Female	Fish	2	5.3	2.0	38.0	10S
16	C	Female	Worm	4	4.4	2.5	28.4	16AB
	D	Female	Worm	12	6.3	3.3	25.0	16AE
	E	Female	Worm	3	5.3	3.7	29.5	16U
	G	Male	Worm	4	3.3	1.1	27.3	10Z
	H	Male	Worm	4	6.5	3.8	33.8	10N
	J	Male	Fish	2	2.6	1.2	28.6	16X
	K	Female	Worm	3	4.9	3.9	33.8	16AF
	M	Male	Fish	6	6.5	4.5	28.3	10Y
	P	Female	Fish	12	5.0	1.4	37.5	10X
	R	Female	Fish	5	3.0	1.3	28.4	16G
	S	Female	Fish	2	4.9	1.4	33.6	10L
	T	Female	Fish	6	4.9	2.5	36.1	10W
	U	Male	Worm	5	6.8	4.3	35.1	16E
	V	Female	Fish	1	4.5	3.7	32.1	16W
	W	Male	Worm	6	8.0	4.3	32.1	16V
	X	Male	Worm	3	6.6	5.2	28.6	16J
	Y	Male	Fish	1	4.3	2.4	39.5	16T
	AB	Male	Fish	5	4.1	1.6	28.4	16C
	AD	Female	Fish	2	3.3	2.1	31.1	10Q
	AE	Male	Fish	2	8.3	4.7	25.0	16D
	AF	Female	Fish	1	6.0	3.3	33.8	16K
	AH	Male	Fish	1	4.6	1.9	34.9	16D
	AJ	Female	Worm	5	7.0	5.8	32.0	10M

Note: Pair NND "Indiv."= individual with smallest Pair NND.

slightly smaller for worm-fed (30.7 cm) than fish-fed animals (33.5), and for litter 16 snakes (31.4 cm) than litter 10 (34.0 cm). Pair NNDs were nearly identical between sexes (males 32.8 cm, females 32.1 cm). Again, means did not differ significantly using a t-test.

Cluster Analysis. As each cluster is formed, it is used as a single entity in all further comparisons in this analysis. Therefore, clusters consisting of a subject and a previously joined pair are here referred to as triads.

Four specific pairs and 3 triads emerged as consistent groups in a density-linkage cluster analysis, as summarized in Table 6.4. All of these primary clusters consisted of unfamiliar animals, and contained at least one animal on each diet. Two pairs and the associated 2 triads consisted of animals of the same litter (all litter 16), but 3 of 4 pairs and all 3 triads were mixed-sex.

Overall Nearest Neighbors (ONN)

Among animals fed fish, pairs of nearest neighbors across all readings were significantly more often composed of animals on opposite diets (baserate 0.38, trials 14, p=0.00003). This was true within litters with the exception of litter 16 worm-eaters, which did not show a significant preference for animals on either diet (litter 10 fish: 0.29, 4, p=0.007; litter 10 worm: 0.21, 3,

Table 6.4: Description of the strongest clusters of *T. sirtalis* generated by density linkage cluster analysis from Pair Nearest Neighbor Distance. (N = 304 observations, mean NND = 32.5 cm).

Pair/Triad	Litter	Subject	Sex	Diet	Housing Group	Mean Distance
1 / 3	16	D	Female	Worm	12	25
1 / 3	16	AE	Male	Fish	2	
3	16	AH	Male	Fish	1	34.9/50.1
2 / 1	10	Z	Female	Worm	3	27.3
2 / 1	16	G	Male	Worm	4	
1	16	R	Female	Fish	5	33.3/28.4
3	10	Y	Male	Worm	5	28.3
3	16	M	Male	Fish	6	
4 / 2	16	C	Female	Worm	4	28.4
4 / 2	16	AB	Male	Fish	5	
2	16	E	Female	Worm	3	29.5/33.8

Note: Pair/Triad number reflects tightness of cluster in descending rank order. NND/NND for Triads refers to the mean NND of the third snake to each of the pair associated with, in order as listed above. Housing group "12" = individually housed.

p=0.0093; litter 16 fish: 0.45, 10, p=0.0003; litter 16 worm: 0.59, 13, p=0.5). There was also a non-significant tendency for males to be nearer members of the opposite sex (baserate 0.51, trials 19, p=0.098). These results are summarized in Table 6.5.

ONN's were significantly more likely to be familiar (baserate 0.13, trials 37, p<0.019). This can be seen in housing groups 2 (baserate 0.19, trials 7, p=0.033), 5 (baserate 0.11, trials 4, p=0.033), and 6 (baserate 0.08, trials 3, p=0.00073). Neither of the solely worm-fed groups showed this trend. Considering the small number of subjects in the mixed diet group 6 (4 familiar animals) makes this finding all the more striking. Group "12," individually housed animals, were not familiar with any other snakes and were not considered in this comparison.

Incidence of Shared Shelters

These data generated 6 pairs with 4 co-occurrences each (snakes as baserate 0.026, observations as trials 8, p<0.00001), and 13 pairs with 3 co-occurrences each (baserate 0.026, trials 8, p=0.00003) across the 8 readings. Since 91 possible pairings occurred at a rate significantly above that of chance (p<0.0009), which were 2 or more pairings of the same individuals, the following comparisons were limited to only those of 4 (6 pairs) or 3 (13 pairs).

Table 6.5. Overall Nearest Neighbor comparisons of *T. sirtalis* in arena aggregation: Frequency that the individual with the least mean separating distance to each subject was of the Same or Other (opposite) Diet, Litter, and Sex, and whether Familiar or not.

	N	Same	Other
Diet: Fish	24	12	12*
Worm	14	5	9
(total)	38	17	21
Litter: 10	15	3	12
16	23	15	8
(total)	38	18	20
Sex: Female	19	9	10
Male	19	7	12
(total)	38	16	22
Familiar		Yes	No
Group: 1	8	1	7
2	8	3*	5
3	5	0	5
4	3	0	3
5	8	3*	5
6	4	2*	2
"12"	2	-	-
(total)	38	9*	27

Note: "*" denotes binomial test $p<0.05$, group "12" are individually housed.

The most frequently occurring pairs as judged by shelter use (those with 4 co-occurrences) are shown in Table 6.6. These pairs consist of a) same diet: 3 (all worm-fed), opposite diet: 3, b) same sex: 3 (all female), opposite sex: 3, c) same litter: 2, opposite litter: 4, and d) familiar: 1, unfamiliar: 5. Thus, these pairs tended to consist of unfamiliar females of different litters. It may be noted that 3 individuals occurred in more than one of these 6 pairings. Also, the familiar animals were in group 5, shown to prefer familiars in ONN comparison above.

A similar comparison of the pairs co-occurring 3 times consists of a) same diet: 4, opposite diet: 9; b) same litter: 8 (all 16's), opposite litter: 5; c) same sex: 10 (7 female), opposite sex: 3; and d) familiar: 1, unfamiliar: 12 (Table 6.7). The apparent preference here for unfamiliar animals is confounded by 7 individuals that occur in multiple pairs within this comparison and 3 others that are found in the previous comparison of pairs occurring 4 times (refer to Table 6.6).

Many animals are present in multiple pairings, 17 snakes comprising all 19 pairs described above. If each of these 17 snakes are compared with the type of snake they are most often paired with, one finds that litter 16 tend to be highly represented and associated with

Table 6.6. Descriptions of the six pairings of *T. sirtalis* found under the same shelter in 4 of 8 observations of arena aggregation.

Pair	Litter	Subject	Sex	Diet	Group
1	10	P	Female	Worm	5
	16	AD	Female	Fish	2
2	10	P	Female	Worm	5
	16	AJ	Female	Worm	5
3	10	Y	Male	Worm	5
	10	Z	Female	Worm	3
4	10	Z	Female	Worm	3
	16	G	Male	Worm	4
5	10	Z	Female	Worm	3
	16	AB	Male	Fish	5
6	16	S	Female	Fish	2
	16	AJ	Female	Worm	5

Table 6.7. Descriptions of the 13 pairings of *T. sirtalis* found under the same shelter in 3 of 8 observations of arena aggregation.

Pair	Litter	Subject	Sex	Diet	Group
1	10	P	Female	Worm	5
	16	C	Female	Worm	4
2	10	P	Female	Worm	5
	16	S	Female	Fish	2
3	10	Q	Female	Fish	2
	16	AD	Female	Fish	2
4	10	Y	Male	Worm	5
	16	W	Male	Worm	6
5	10	Z	Female	Worm	3
	16	R	Female	Fish	5
6	16	C	Female	Worm	4
	16	S	Female	Fish	2
7	16	C	Female	Worm	4
	16	Y	Male	Fish	1
8	16	C	Female	Worm	4
	16	AD	Female	Fish	2
9	16	G	Male	Worm	4
	16	R	Female	Fish	5
10	16	G	Male	Worm	4
	16	AB	Male	Fish	5
11	16	J	Male	Fish	2
	16	X	Male	Worm	3
12	16	T	Female	Fish	6
	16	Y	Male	Fish	1
13	16	AF	Female	Fish	1
	16	AJ	Female	Worm	5

siblings, and females are also commonly associated. This comparison is illustrated in Table 6.8.

Six snakes shared shelters with 8 or fewer other individuals over the course of the observations, with subject 16AE co-occurring under a shelter with only 4 of the other 37 snakes. The other 5 snakes with such low ISS values were all from litter 10, 3 of which (along with 16AE) had been found in the open in 4 to 6 of the observations. Four of these animals were also never found under the same shelter twice, indicating that these animals may have been more exploratory than some of their compatriots.

Discussion

Thamnophis s. sirtalis used in this experiment were not found to be highly aggregating animals, forming significantly large clusters in only 3 of the 8 observations. Rather, subjects tended to distribute themselves throughout the arena in small groups, with individuals showing significant site fidelity towards one to three different shelters. This lack of strong aggregation is in contrast to the earlier work with T. butleri, in which animals formed large clusters repeatedly under one or two specific preferred shelters, but could be due to species differences (Lyman, 1990). However,

Table 6.8: Frequency that T. sirtalis shared shelters with animals that were more often of the Same or Other diet, litter, or sex, or showed no bias (both diets, litters, or sexes equally represented).

	N	Same	Other	No Bias
Diet: Fish	9	2	6	1
Worm	8	2	4	2
(total)	17	4	10	3
Litter: 10	4	0	3	1
16	13	10	2	1
(total)	17	10	5	2
Sex: Female	11	8	2	1
Male	6	3	1	2
(total)	17	11	3	3

earlier studies using T. sirtalis had also demonstrated stronger clumping than in the current study. It is possible that the use of older, experienced snakes explains the smaller groups than seen in neonate T. sirtalis also presented with 8 shelters (Burghardt, 1983). The animals in the present study may have been more exploratory, possibly searching for food, than newborns in a similar situation. Heller and Halpern (1982a) used adult male T. sirtalis that showed significant aggregation and site fidelity, but only had 4 shelters to choose from. The more diffuse patterns seen in the current study may have been a direct result of the greater number of sites to choose from, combined with a high level of exploratory activity. Also, Schwartz (1989) found that T. sirtalis from a Michigan population were more active and less often under covers than animals from a Wisconsin population, suggesting that this behavior is characteristic for this population. Additionally, being juveniles, any accentuation of aggregatory urges for reproductive purposes may not yet have been in effect.

There is also some possibility that temperature at gestation may affect the behavior of snakes (Burger, 1990, 1991; Burger, Zappalorti and Gochfeld, 1987), and these animals had been subjected to relatively warm temperatures (30 C) during pre-natal development. As discussed in greater detail in Chapter 2, it is not believed that this

had any detrimental effects upon the health of the subjects, but it is possible that there was an effect upon activity levels and amount of exploratory behavior (Burger, 1990).

Although the groups formed by T. sirtalis in this study were not large, they were consistant and demonstrated certain patterns regarding the types of individuals most closely associated with. In order to get a complete picture of occurrences, all comparisons described above should be considered together since each has a limited focus within itself. For example, the nature of a cluster analysis will place each individual in only one position, here generating discrete bimodal clusters with individuals or existing other clusters. In the comparison of shared shelters, however, one individual may occur in multiple associating pairs, allowing a view of the type of snake preferred as opposed to specific individuals. There were no contradictions between the comparisons made, and several results corroborate each other and indicate particular overall patterns of association.

Cluster analysis and Incidence of Shared Shelters (ISS) comparisons both indicated an effect of litter in association patterns. Members of litter 16 were frequently found in associating pairs of animals. Snakes in litter 10 seemed to be more active, occurring in the

open more often than those in litter 16, which perhaps accounts for their lower incidence in associations with other snakes. Alternatively, litter 16 animals may have shown a stronger preference to be near other snakes. In any case, litter 16 animals were significantly more often associated with siblings than with members of litter 10. This may be evidence of kin recognition in T. sirtalis, and parallels the findings in T. butleri (Lyman, 1990).

Meanwhile, although cluster analysis showed groups consisted of mixed sexes, ISS comparisons showed a preponderance of females, while ONN (Overall Nearest Neighbors) displayed a slight tendency for males to be nearer members of the opposite sex. These results combine to illustrate a case in which females may be more inclined to aggregate with each other, whereas males seek out females when associating with other snakes. This corresponds to patterns seen in adult T. sirtalis in the wild, in which gestating females are found to aggregate (e.g., Aleksiuk, 1977; Finneran, 1953; Gregory, 1975) whereas males show such inclinations mainly in pursuit of reproductively active females (e.g., Crews and Garstka, 1982). This would tend to show an overall pattern of mixed sex groups, somewhat biased in favor of females, that is seen here.

Only weak tendencies can be discerned regarding effects of diet in association patterns. ONN comparison

demonstrated that fish fed animals were often nearer animals on the opposite diet, while ISS showed a slight pattern of worm-eaters associating with animals on the same diet. This combines to show a weak preference to associate with animals feeding upon worms, more noticable in individuals fed fish. The response of fish-eaters appears to match the pattern seen in earlier testing with T. butleri (Lyman, 1990), but worm-eaters correspond more with the site choice testing reported in Chapter 5. The latter testing had shown a weak preference for subjects to associate with sites marked with feces of conspecifics fed a familiar diet, with an overall bias in favor of worm-fed feces cues. However, the effects are so slight that it is difficult to fully interpret these results without further investigation.

Among the interesting results seen in ONN comparisons was the occurrence of familiar animals in significantly close proximity. Due to the multiple housing groups, there were far more unfamiliar animals in the arena to interact with than there were familiar ones. However, animals in 3 housing groups were found to be associating with familiar animals at a significant level of frequency. This differs strongly from the results of work with T. butleri which, despite similar circumstances, had shown a significant lack of association with familiars. This phenomenon therefore deserves more detailed discussion.

Neither group consisting of only worm fed animals (groups 3 and 4) was shown to associate with familiar animals, but both of these groups were small in number (only 5 and 3 members, respectively) so it is not possible to interpret the association. Both groups consisting of fish fed animals (groups 1 and 2) had full complements of 8 snakes each, and one of these groups displayed association with familiars (group 2, $p=0.033$). This implies that fish diet alone does not predict for this behavior. The most interesting groups were those that contained animals on each of the two diets (groups 5 and 6). Group 5 contained a full complement of 8 animals, 5 of which repeatedly appeared in groups produced in other comparisons, and showed a significant attraction to familiars ($p=0.033$). Group 6 only contained 4 animals at the time of this experiment, yet these animals showed the strongest affinity for familiars of any group ($p=0.00073$). Neither of these groups showed any effect of diet upon this phenomenon.

Yeager and Burghardt (in press) have demonstrated that T. radix prefer to associate with familiar non-competitors over animals that they had competed with for food, also supporting the possibility of individual recognition in snakes. Thamnophis butleri in aggregation had preferred unfamiliar animals, inviting the possibility that snakes generalized, such that familiarity implied competition

regardless of actual experience. This appears not to be the case in the T. sirtalis in this experiment. Effects of competition upon association have not yet been ascertained in this species, but it is possible that T. sirtalis do not confuse familiarity with competition without prolonged experience. An effect of familiarity upon association patterns does suggest a capacity for individual recognition.

A preference for familiar conspecifics has been demonstrated in several species of animals, usually in the context of determining close kin. In many species, familiarity with nestmates virtually assures close relatedness since littermates are always full siblings (e.g., Dewsbury, 1988a. 1988b; Ferkin, 1990). However, garter snakes probably diperse shortly after birth, and even members of a given litter may show multiple paternity (Schwartz, 1989), so a reliance upon familiarity for kin recognition would be of limited utility. Thamnophis sirtalis of one litter in this experiment demonstrated a preference for siblings that did not depend on familiarity, showing that these snakes may be able to determine relatedness without direct familial experience. It seems that the behavior of T. sirtalis is therefore congruent with the phenomenon of phenotype matching, in which animals compare others to a template based upon themselves or "known" relatives rather than rely solely on

familiarity to recognize kin (Ferkin, 1990; Holmes and Sherman, 1983).

The association of snakes with familiar animals may still be related to kin recognition, however. Belding's ground squirrels showed an ability to discriminate (and respond positively to) offspring of unrelated animals they had been reared with in addition to an ability to recognize unfamiliar siblings (Holmes, 1986a, 1986b). This indicated that animals could use experience to modify an otherwise innate ability. Neonate rattlesnakes, C. viridis, are known to aggregate, trailing each other to hibernacula (Graves et al., 1986). Newly born garter snakes probably spend a relatively brief period of time near their littermates and mother before dispersing into the environment, but the details of such movements are unknown. However, it may be long enough to establish some familiarity with one's littermates. Also, even if some littermates have different fathers, they are more likely to be closer kin than any non-littermates encountered. In this scenario, it is entirely possible that a preference in aggregation for familiar animals and for siblings may be due to an interactive effect of these factors upon kin recognition.

It is, of course, also possible that the two preferences are due to unrelated underlying mechanisms. Territorial salamanders have been shown to prefer areas

marked with fecal pellets of familiar neighbors, and respond with less aggressive behavior than toward cues of unfamiliar animals (e.g., Jaeger, 1981, 1986). Although T. sirtalis are not known to be territorial, the subjects in this study assorted themselves into several relatively scattered small groups rather than the large, dramatic aggregations seen in some other work. Little is known about the home range patterns of wild garter snakes. It is possible that wild T. sirtalis may become acquainted with other individuals with overlapping home ranges, and show preferences to associate with those familiar animals in subsequent aggregation behavior, regardless of any inclinations based on other factors such as kinship, sex, or diet.

In conclusion, Thamnophis s. sirtalis showed low rates of aggregative behavior such that animals dispersed through the testing arena in small groups. This may be due to relatively high activity levels resulting in frequent observations of snakes moving about in the open. Association patterns within groups included an effect of relatedness, such that one of the litters were more frequently in groups with siblings, and a preference for familiar individuals that was not affected by diet. There was some evidence that females may be more prone to aggregate, but that males in groups preferred to be near members of the opposite sex. Any effect of dietary

experience was weak, but appeared to be in the direction of a preferred association with animals fed worms, especially in individuals themselves fed on fish. Snakes in an homogenous habitat showed patterns of association corresponding to characteristics of other individuals rather than the environment, demonstrating that there is a social element, possibly including individual and/or kin recognition, to the aggregation of this species of garter snake.

CHAPTER 7

COMPARISON AND DISCUSSION

Introduction

This chapter will begin with a brief review of the work described previously. Possible interactions between key variables will then be presented. The significance of these findings and a comparison with previous results with other species will occupy the remainder of this text.

Overview

Neonate snakes are known to show immediate preferences for certain prey. Nevertheless, these preferences can be modified through experience (Arnold, 1978; Burghardt, 1969; Fuchs and Burghardt, 1971). The research that is reported in this dissertation was designed in part to consider the possibilty that these prey preferences may be manifested in site choice and aggregative behavior. In so doing, factors related to attraction seen in other species, such as relatedness or familiarity, have been taken into consideration.

As was outlined in the introduction, it is possible that given their high degree of reliance on chemical cues (e.g., Burghardt, 1970; Heller and Halpern, 1982a, 1982b;

Wilde, 1938), snakes could be using conspecific feces as cues for aggregation (Allen, Burghardt and York, 1984; Dundee and Miller, 1968; Porter and Czaplicki, 1974; Scudder, Stewart and Smith, 1980). Further, such animals may be able to gather information regarding the diets of other animals in the area when choosing these sites (Burghardt, 1990; Lyman, 1990; Walls et al, 1989). Snakes may also be able to recognize kin, and show patterns in aggregation based on relatedness or familiarity (Lyman, 1990).

To investigate the importance of conspecific cues in aggregation behavior, an experimental program was designed in which neonate garter snakes were (1) raised in group housing situations on diets of fish or earthworm, (2) tested for prey preference, and subsequently (3) site choice based on prey and conspecific cues. They were then observed in (4) arena aggregations. This design allowed the effects of diet, prey preference, familiarity, litter, and sex to be evaluated in site choice and aggregative behavior.

The animals used were eastern garter snakes, Thamnophis sirtalis sirtalis, born to 2 gravid females collected in Michigan. This species was utilized for a variety of reasons. Among them, it has large litters and is easily managed in the laboratory. It is a dietary generalist with feeding habits similar to those of T.

radix, used in earlier work of this nature (Carpenter, 1952a; Burghardt, 1990; Burghardt and Lyman-Henley, in preparation). Thamnophis sirtalis has also been reported to engage in aggregation behavior outside of hibernation and reproductive periods (Burghardt, 1983; Heller and Halpern, 1982a; Schwartz, 1989).

General methodology was discussed in Chapter 2, including details regarding the origin of subject animals, housing, feeding, etc. The ecology of the species was also summarized.

Chapter 3 concerned growth and found that the main difference in both mass and snout-vent length was between the litters, with litter 16 snakes being longer and heavier than litter 10 animals throughout the study, apparently due to heritable differences. An interactive effect of sex with litter was found regarding snout-vent length at the final measurement such that males were larger than females in litter 16, whereas females were the larger sex in litter 10. Assuming that this population normally demonstrates a sexually dimorphic growth rate, as has been previously found for this species (Carpenter, 1952b; Crews et al, 1985), the limited amount of food available to the animals may have kept females from out-growing males (Scudder, 1983).

Overall, the two diets bore little relationship to differences in animal size or growth. It seems that the

addition of calcium-phosphorus supplements to the worm diet negated any deficiency of these minerals compared to that contained in fish, allowing very similar growth rates between diets (Burghardt, 1990; Lyman, 1990; Scudder-Davis and Burghardt, 1987). Since growth is assumed to be dependent on relatively good health and nutritional intake, this lack of difference in growth between diets should translate into a negligible difference between health of the subjects. This in turn should control for effects of general health upon the other measures used in this research, thereby avoiding any aversive reactions of the subjects toward prey stimuli due to nutritional deficiencies (Partridge and Maclean, 1981).

In Chapter 4 the chemical prey preferences of the subjects were investigated. It was hypothesized that (1) T. sirtalis, being a dietary generalist, would respond without prior experience to surface extracts of both fish and earthworm. In tests of chemical prey preference prior to feeding experience, the T. sirtalis in this experiment gave high responses to both fish and worm prey surface stimuli over a water control, but a preference for worm was revealed by the higher rate of attack toward that stimulus.

It was also hypothesized that (2) after experience this species would favor the chemical prey cues of familiar prey. An influence of experience was

demonstrated in that fish fed animals moved from a moderate worm preference to a weak fish bias, although worm fed animals showed no change in bias.

Strong effects of litter upon chemical prey preferences were also found. Litter 10 showed a strong initial preference for worm cues while litter 16 showed no preferences. In the final test, litters still showed great differences in preference, with litter 16 fish-eaters now demonstrating a strong preference for fish cues, while litter 10 fish-eaters retained a worm preference. The animals also showed different levels of responsiveness, litter 10 snakes giving 15 of the 16 total attacks in the initial test. There was a mild effect of sex on preferences such that females showed greater preferences for worm while males demonstrated more of a fish bias, even in worm fed animals.

There were interesting patterns of response to chemical stimuli related to housing group. In situations where all animals in a group ate the same prey, mean responses were highest toward that stimulus. The two groups that contained animals on each diet, however, showed higher responses for worm stimuli in group 5 and fish stimuli in group 6, regardless of individual diets. This may be weak evidence that there could be some influence of the diets of familiar conspecifics upon prey preference, external to one's own experience.

Chapter 5 investigated preferences of the subjects to sites marked with prey or conspecific cues. It was hypothesized that (3) site choice would be in favor of the familiar prey cues. This would make ecological sense, since prey should be more likely to be found where its chemical cues are strongest. The site choice test based on prey surface-chemical cues revealed an overall preference for sites marked with worm cues, strongest in those animals fed worm.

However, it was postulated that (4) site choice based on conspecific feces should be in favor of cues based on the unfamiliar, nonpreferred, prey. This was based on the previous research with T. radix and T. butleri that indicated that rather than associating with feces of other animals feeding on the same preferred prey, garter snakes may avoid such areas to reduce competition for that resource (Burghardt and Lyman-Henley, in preparation; Lyman, 1990). Prior to any experience with feeding (or defecation), these subjects showed a significant bias in favor of feces carrying worm cues. The final feces choice test, however, shows a general trend for animals to prefer the same sites based on feces as they did regarding prey surface cues, again mostly worm-based. What changes occurred between tests were most often in favor of familiar prey cues.

This response supports the possibility of information transfer (or extraction) in which animals may choose sites (foraging areas, etc.) based on conspecific feces carrying preferred prey cues. Thamnophis sirtalis females preferred sites marked with fish-based feces cues, while males showed a preference for worm based cues.

It was noted that T. sirtalis seemed to show only a relatively weak interest in the test stimuli, several individuals showing a preference based on the side of the tank rather than the test stimuli. This limited the number of snakes showing strong preferences based on stimuli, making interpretation of the results that much more difficult. It is possible that external environmental conditions may have been more important to these snakes than to the other species used in the past, such as the difference in light levels between sides of the test containers. Similarly, disturbance from the investigator always came from the same side of the tank, and animals may have been reacting defensively (Herzog, Bowers and Burghardt, 1989).

Finally, in Chapter 6 the T. sirtalis housed with conspecifics or alone were observed for aggregative behavior in a single large arena. It was hypothesized (5) that animals would aggregate preferentially with unfamiliar littermates on the same diets. Earlier work with T. butleri had demonstrated a preference for animals

on unfamiliar diets, but had shown preferences for sites marked with feces from conspecifics fed the unfamiliar diet as well. Thamnophis sirtalis had rather preferred sites marked with feces based on the familiar diet, suggesting the current hypothesis.

Thamnophis s. sirtalis tended to distribute themselves throughout the arena in small groups, showing site fidelity towards up to 3 different shelters. Earlier studies using T. sirtalis had demonstrated stronger clumping and site fidelity than the current study, possibly due to the use of snakes of different ages and with different numbers of shelters available. There is also evidence that animals from this Michigan population may be more active than populations from other areas (Schwartz, 1989), and relatively high gestation temperatures (discussed in Chapter 2) have also been shown to increase activity levels of snakes (Burger, 1990, 1991).

An effect of litter was demonstrated in association patterns such that members of litter 16 were frequently found in associating pairs of animals, while litter 10 animals seemed to be more active, occurring in the open more often than those in litter 16. Schwartz (1989) also found differences between litters regarding aggregation behaviors, and reported a heritable basis for these tendencies. Litter 16 animals were significantly more

often associated with siblings than with members of litter 10, which may be evidence of kin recognition in T. sirtalis via phenotype matching. These results also illustrate a case in which females may be more inclined to aggregate with each other, while males seek out females when associating with other snakes. Effects of diet combine to show a weak preference to associate with animals feeding upon worms, more noticeable in those individuals fed fish.

Animals in 3 of the 6 housing groups were found closely associating with familiar animals at a significant rate. One of the 2 groups consisting of fish fed animals displayed an association with familiars, implying that fish diet alone does not predict for this behavior. Both groups containing animals on each of the two diets (groups 5 and 6) showed significant attraction to familiar animals. Neither of these groups showed any effect of diet upon this phenomenon.

It is possible that a preference in aggregation for familiar animals and for siblings may be due to an interactive effect of these factors upon kin recognition. Alternatively, these preferences may be due to unrelated underlying mechanisms, such as those seen in territorial salamanders that prefer familiar neighbors (Jaeger, 1981).

Interactions

Some of the patterns emerging between the different experiments have been mentioned in context of discussion of the results of a specific test. At this time, I will compare the results of these tests more formally and determine any unifying patterns.

The single most striking pattern visible across all tests is that of a difference between animals in the two litters. Litter 16 animals tended to prefer fish cues, strengthened by experience with that prey. They were larger animals than those in litter 10 throughout the study. Meanwhile, the smaller litter 10 animals showed a strong preference for worm cues, only slightly weakened by experience solely with fish. Litter 10 snakes were also more reactive to chemical stimuli and more active in the aggregation arena than litter 16 animals. Upon closer examination, it appears that these differences in different tests tie together in a sensible fashion.

Burghardt (1975) discusses differences in chemical prey preferences within litters of T. sirtalis, pointing out the adaptive value of having a few individuals in a litter which readily respond to less common food items. Such prey preference polymorphism may prohibit overspecialization within a litter upon a particular prey type, or limit depletion of the prey animals in an area. It is possible that a "generalist" species, such as T.

sirtalis, may demonstrate a theoretically similar phenomenon, actually being comprised of complexes of different adaptive strategies. Few environments, if any, are truly homogeneous. Foraging strategies which are specialized for utilization of different microhabitats within the general habitat range of an animal may confer an adaptive advantage. This would result over time in heritable patterns of behavior that differ between lines of descent such that certain groups of individuals would have greater foraging specializations for particular habitats than others. This may be exemplified by the two litters of t. sirtalis in this research. Each show different innate preferences and behavior patterns that suit them to forage for different prey types in a heterogenous environment (but with enough flexibility not to be stranded if their preferred prey is unavailable).

The strong differences between the two litters in this study also illustrate the importance of large sample sizes when attempting to form generalizations about a species, or even a population. If both litters had demonstrated more similar behavior patterns, it would have been easily assumed standard for this population of T. sirtalis. Luckily, they did not, and some intriguing possibilities were thereby discovered.

It is desirable to further the above comparisons in search of any other clues towards the deciphering of these

behaviors. To accomplish this, variables reflecting each factor tested here were compared across experiments using Pearson correlation and contingency tables. In this way any relationships between such factors as animal size, chemical response, site choice, and aggregative patterns may be determined.

Correlation Matrix

A correlation matrix was utilized to describe basic relationships between the variables used within and between different experiments.

Methods

Measures. Fish - Worm (F-W) scores in each Chemical Prey Preference test and Site Choice test were utilized. Mean Tongue-Flick Attack Score (TFAS) toward worm and fish surface extracts for each Chemical Prey Preference test were also used as a more absolute measure of responsiveness to each stimulus.

The initial and final value of snout-vent length (SVL1, SVL3) and mass (Mass1, Mass3) were both included as measures of size. Similarly, there was no one dependent measure for aggregation behavior, so the mean distance to each subject's nearest neighbor across all trials (NND)

was used, as well as the mean distance to the nearest associated individual (pair nnd= PNND).

Analysis. Pearson product-moment correlations were run utilizing the measures described above. Analyses were run using all subjects across all variables, and on animals used only in the aggregation test. In both cases, analyses were performed with animals separated by diet as well as pooled. NND and PNND were only utilized in tests using aggregation tested animals. Since analyses run with aggregation tested animals conservatively paralleled results of analyses with all animals, further breakdowns by litter, diet, and sex were run using only aggregation tested snakes. Correlations were performed using Statgraphics 3.0 (STSC, 1988).

Results

Pearson correlation analysis supports comparisons already made between tests, and suggests some connections between factors that had not been noticeable before. Table 7.1 summarizes correlation coefficients, reporting R values with a significance of $p<0.10$ for both diet groups and all snakes. Due to the large number of variables utilized, only significance levels of $p<0.001$ will be discussed to avoid Type I errors.

Table 7.1: Pearson correlation coefficients of $p < 0.1$ among variables across experiments, using aggregation-tested *T. sirtalis* pooled (N=38), on fish diet (N=24), and on worm diet (N=14).

Var.	diet	Worm1	Fish1	F-W1	Worm2	Fish2	F-W2	Feces Choice1	Feces Choice2	Prey-surface Choice	Mass1	Mass3	SVL1	SVL3	NND
Fish1	all	.393+													
	fish	.462+													
	worm	-													
F-W1	all	-.823**	-												
	fish	-.858**	-												
	worm	-	-												
Worm2	all	.457*	-	-.317											
	fish	.426+	-	-											
	worm	.523	-	-											
Fish2	all	-	-	-	-										
	fish	-	-	-	-										
	worm	-	-	-	-										
F-W2	all	-.386+	-.297	-	-.594**	.606**									
	fish	-.447+	-.370	-	-.734**	.525*									
	worm	-	-	-	-	.694*									
Feces Choice1	all	-	-	-	-.276	-	.289								
	fish	-	-	-	-	-	.472+								
	worm	-.595+	-	-	-	-	-								
Feces Choice2	all	-	-	-	-	-	-	-							
	fish	-	-	-	-	-	-	-							
	worm	-	-	-	-.486	-	-	-							
Prey-surface Choice	all	-	-	-	-	-	-	-	-						
	fish	-	-	-	-	-	-	-	-						
	worm	-	-	-	-	-	-	-.593+	-						
Mass1	all	-.540	-	.503*	-	-	-	-	-	-					
	fish	-.567*	-	.539*	-	-	-	-	-	-					
	worm	-.498	-	.501	-	-	-	-	-	-					
Mass3	all	-.383+	-	.314	-.302	-	-	-	.273	-	.331+				
	fish	-.452+	-	-	-.354	-	.414+	-	-	-	-				
	worm	-	-	-	-	-	-	-	-	-	-				
SVL1	all	-.758**	-	.633**	-.395+	-	.429*	-	-	-	.754**	.341+			
	fish	-.694**	-	.618*	-.469+	-	.388	-	-	-	.794**	.442+			
	worm	-.852**	-	.672*	-	-	.479	.515	-	-	.706*	-			
SVL3	all	-.456*	-	.403+	-.397+	-	.343+	-	-	-	.437*	.723**	.576**		
	fish	-.387	-	.366	-.497+	-	.474+	-	-	-	.441+	661**	.621*		
	worm	-.555+	-	.424	-	-	-	-	-	-	-	.833**	.503		
NND	all	-	-	-	-	-	-	-	-.347+	-	-	-	-	-	
	fish	-	.360	-	-	-	-	-	-.444+	-	-	-	-	-	
	worm	-.575+	-	-	-	-	-	.703*	-	-	-	-	-	-	
PNND	all	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	fish	-	.350	-	-	-	-.358	-	-	-	-	-	-.367	-	-
	worm	-	-.469	-	-	-	-	-	-.468	.583+	-	-	-	-	-

Note: + = p<0.05, * = p<0.01, ** = p<.001.
Abbreviations: Worm1=initial worm Tongue-flick attack score (TFAS), Fish1=initial fish TFAS, F-W1=initial fish-worm TFAS response, Worm2=final worm TFAS, Fish2=final fish TFAS, F-W2=final fish-worm TFAS response, Feces Choice1=initial feces-based site choice, Feces Choice2= final feces-based site choice, Prey-surface Choice= prey surface-chemical based site choiceMass1=initial mass, Mass3=final mass, SVL1=initial snout-vent length, SVL3=final snout-vent length, NND=nearest neighbor distance, PNND=NND between pairs of individuals.

First, among prey preference scores note that the initial responses to worm and fish extracts are correlated for those animals later fed fish ($R= 0.462$, $p<0.05$), but not worm. However, in the final test responses to the two stimuli were not correlated at all, either regarding stimulus scores or F-W scores.

In addition to being correlated with other measures of size, length was correlated with responses to chemical cues, specifically those toward worm stimuli. These correlations indicate a higher responsiveness to worm cues in smaller animals, corresponding to the findings that smaller litter 10 animals were strong worm-responders.

Measures of association (NND, PNND) did not significantly correlate with each other, reflecting that animals found physically close to other snakes in general did not necessarily engage in close association with particular other individuals.

Table 7.2 similarly summarizes Pearson correlation coefficients of the analyses using aggregation tested animals, comparing animals of different litters to the overall results. The patterns are similar to those comparing diet, with several fewer significant correlations, but one noteworthy addition. Litter 16 snakes that showed final preferences for sites marked with fish-based feces cues had smaller NNDs ($R= -.688$, $p<0.001$).

Table 7.2. Pearson correlation coefficients of $p < 0.1$ among variables across experiments, using aggregation-tested *T. sirtalis* pooled (N=38), and separated by litter (litter 10 N=15, litter 16 N=23).

Var.	diet	Worm1	Fish1	F-W1	Worm2	Fish2	F-W2	Feces Choice1	Feces Choice2	Prey-surface Choice	Mass1	Mass3	SVL1	SVL3	NND
Fish1	all	.393+													
	10	-													
	16	-													
F-W1	all	-.823**	-												
	10	-.837**	-												
	16	-.419+	.758**												
Worm2	all	.457*	-	-.317											
	10	-	-	-											
	16	-	-	-											
Fish2	all	-	-	-	-										
	10	-	-	-	-										
	16	-	-	-	-										
F-W2	all	-.386+	-.297	-	-.594**	.606**									
	10	-	-	-	-	.875**									
	16	-	-	-	-.538*	.590*									
Feces Choice1	all	-	-	-	-.276	-	.289								
	10	-	-	-	-	-	-								
	16	-	-	-	-	-	-								
Feces Choice2	all	-	-	-	-	-	-	-							
	10	-	-	-	-	-	-	-							
	16	-	-	-	-	-	-	-							
Prey-surface Choice	all	-	-	-	-	-	-	-	-						
	10	-	-	-	-	-	-	-	-						
	16	-	-	-	-	-	.369	-	-						
Mass1	all	-.540	-	.503*	-	-	-	-	-	-					
	10	-	-	-	-	-	-	-	-	-					
	16	-.562*	-	-	-	.360	-	-	-	-					
Mass3	all	-.383+	-	.314	-.302	-	-	-	.273	-	-				
	10	-	-	-	-	-	-	-	-	-	-				
	16	-	-	-	-	-	-	-	-	-	-				
SVL1	all	-.758**	-	.633**	-.395+	-	.429*	-	-	-	.754**	.341+			
	10	-.584+	-	.572+	-	-	-	-	-	-	.867**	-			
	16	-	-	-	-	.373	-	-	-	.487+	.670**	-			
SVL3	all	-.456*	-	.403+	-.397+	-	.343+	-	-	-	.437*	.723**	.576**		
	10	-	-	-	-	-	-	-	-	-	.527+	.784**	-		
	16	-	-	-	-	-	-	-	-	-	-	.541*	-		
NND	all	-	-	-	-	-	-	-	-.347+	-	-	-	-	-	
	10	-.633+	-	.561+	-	-	-	-	-	-	-	-	-	.597+	
	16	-	-	-	-	-	-	.413	-.688**	-.373	-	-	-	-	
PNND	all	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	10	-.487	-	.559+	-	-	-	-	-	-	-	-	-	-	.566+
	16	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: + = $p<0.05$, * = $p<0.01$, ** = $p<0.001$.
Abbreviations as in Table 7.1.

Contingency Tables

Due to the diverse nature of the variables and measures used in the different experiments within this research, no single statistical comparison is appropriate. Therefore, I have compared specific variables between each possible pairing of tests using contingency tables in order to view the relationships between experiments.

Methods

A single bivariate measure was used to represent the results of each experiment with the exception of arena aggregation, in which two measures were used.

Size. Animals were rank-ordered by snout-vent length (SVL) at the second observation (91 days of age), when all subjects used in final analyses were living. SVL had been the most consistent measure of size, and was generally consistent between observations. Snakes were scored for contingency tables as being either above or below the median size.

Chemical Prey Preference. Fish-Worm (F-W) scores were used to provide a worm or fish preference for each subject for both Initial and Final tests. F-W scores of 0 (no preference) were not utilized.

Site Choice Tests. As in Chemical Prey Preference testing, Fish-Worm (F-W) scores were used to provide a worm or fish preference for each subject for both Initial and Final tests. F-W scores of 0 (no preference) were again not used.

Aggregation. Two measures were used in comparing aggregation patterns to other experiments. The first was Nearest Neighbor Distance (NNDistance), in which the mean distance between subjects and their nearest neighbors was scored as either above or below the median. The second measure used was Nearest Neighbor Diet (NNDiet), in which the nearest neighbor to each subject over all 8 readings was scored for either a fish or worm diet.

Analysis. Contingency tables were created comparing all possible pairs of tests, using both all-snake comparisons and comparisons by litter, diet and sex. Contingency tables were analyzed using two-tailed Fisher's Exact Tests, since in several cases expected values were below five. This statistic was used in all cases for consistency, and was performed using Statgraphics 4.0 (STSC, 1990).

Results

Considering the low number of significant effects found between experiment measures, results of Fisher's Exact Test of $p<0.10$ will be discussed.

Size vs. Final Chemical Prey Preference. There was a significant relationship between animal size and Final Chemical Prey Preference (2-tailed Fisher's Exact $p=0.017$). Snakes preferring worm cues were more often smaller than median SVL (20 vs. 10 larger animals), whereas animals preferring fish cues were more frequently larger than median size (19 vs. 9 smaller than median SVL). This was extremely noticeable among fish-fed snakes ($p=0.008$), in which animals preferring fish cues were much more frequently larger than median SVL (14 large vs. 4 small) whereas those preferring worm cues were more likely small (9 vs. 3 larger SVL). Although not significant, there was a similar relationship among males ($p=0.054$) such that fish preferrers were more often large (11) than small (5), whereas worm preferrers were more frequently small (8) than large (3).

Size vs. Other Measures. There was a non-significant relationship between animal size and Initial Feces-Based Site Choice among those animals to be fed a worm diet ($p=0.088$), such that smaller animals more often preferred

worm-based conspecific cues (8) than larger animals did (2), whereas the few snakes to prefer fish-based cues were more often larger (4 vs. 1 smaller than median SVL).

There was another non-significant relationship between size and NNDiet ($p=0.08$) among litter 16 animals, such that larger animals were more frequently associated with worm-fed snakes (11 vs. 5 fish-fed snakes), but smaller than median SVL snakes were more often found near fish-fed animals (6 vs. 2 worm-fed).

Final Feces-Based Site Choice vs. Other measures. There were no significant relationships between Feces-Based Site Choice and measures of other experiments, but two cases warrant brief description. There was a relationship with Final Chemical Prey Preference among females ($p=0.09$) in which snakes more frequently preferred the same prey cue in both tests. Those that preferred worm chemical prey cues more often preferred worm-based feces cues (8 snakes vs. 6 preferring fish-based feces cues), whereas those preferring fish chemical cues more frequently preferred fish-based feces cues (9 vs. 2 preferring worm-based feces cues).

There was also a relationship (though not significant) between Final Feces-Based Choice Test preferences and NNDistance ($p=0.08$) in which animals preferring worm-based cues were more frequently less than

the median distance from other snakes (7) than separated by greater distances (3), whereas subjects preferring fish cues were more often farther from other snakes (8) than nearer to them (3).

Prey Surface-Chemical Choice Test vs. NNDiet. There was a significant relationship between these measures among males ($p=0.03$). Male snakes preferring sites marked with worm cues more often were nearer animals feeding on worms (8) than fish (2). Meanwhile, those snakes preferring sites marked with fish cues were frequently associated with other snakes feeding on fish (5) rather than on worm (1).

Final Discussion

Snakes have often been considered the least social of reptiles, long thought to lack complex behaviors (Allee, 1931, 1938; Brattstrom, 1974; Prater, 1933; Wilson, 1975). However, a variety of social behaviors have been documented in snakes, including male combat (Carpenter, 1977; Gillingham, Carpenter and Murphy, 1983), courtship and mating aggregations (Crews and Garstka, 1982; Finneran, 1949), communal nesting (Swain and Smith, 1978), and hibernation aggregations (Aleksiuk, 1977; Duvall, King and Gutzwiller, 1985). Aggregations outside of

reproductive and hibernation periods also occur, but the reasons are not always so clear (Barbour, 1962; Dundee and Miller, 1968; Noble and Clausen, 1936). The experiments reported in this dissertation were intended to investigate some of the mechanisms involved in this little understood behavior.

Patterns Seen in Eastern Garter Snakes

The implications of this research are that the two litters used have different basic strategies for survival. Animals naturally preferring worms may need to be more active foragers, alert to diffuse chemical signals to locate their preferred prey. They will, in general, require more mass of food than counterparts searching out fish due to the differences in water and calcium content between the two prey items (Scudder-Davis and Burghardt, 1987), and compensation for this difference may be incorporated into their behavior. Alternatively, animals preferring fish are searching for a prey that has strong visual cues in addition to strong chemical cues, and are located in fairly specific areas. However, animals with inflexible preferences may perish if the preferred resource is unavailable or sparse, so an ability to adjust responses to prey based on experience is very advantageous. This interaction of innate responses with experience is dynamic and ongoing (Burghardt, 1969).

To continue this line of supposition a little further, the patterns of sociality start making some sense. Litter 10 animals, generally worm-preferrers (and smaller animals), were more active and exploratory, and less inclined to engage in aggregative behavior than their more sedentary counterparts in litter 16. These animals, the larger fish-preferrers, showed some preference to associate with siblings in resting groups. Fish being a somewhat difficult prey to capture, garter snakes are known to be very opportunistic in exploiting sometimes ephemeral sources such as drying pools and fish hatcheries (Carpenter, 1952a; Lagler and Salyer, 1945). This is exactly the group of animals that would be best served by any use of information extraction, and who best to share such information with but one's siblings? On the other hand, earthworms are probably a more evenly distributed prey in the environment (given appropriate conditions of soil type and moisture), and snakes may be better served to forage for them independently, and whenever possible reduce competition for such a resource.

However, there are a few conflicting tendencies, such as those involving sex differences. Females showed a somewhat greater affinity for aggregation than males regardless of diet or litter, and tended to have stronger preferences for worm cues, which contradict the previously outlined "adaptive story." Gravid females are known to

form aggregations in nature, especially for thermal benefits (Aleksiuk, 1977; Fitch, 1960; Gregory, 1975), so this tendency in itself is not too surprising to see even in juveniles. Also, one could assume that it would be more advantageous for females to exploit more constant food sources rather than rich but ephemeral ones, suggesting a worm rather than fish preference. Differential predation between the sexes has apparently not been investigated in studies of wild populations past any differences attributed to size (Carpenter, 1952a; Fitch, 1965). So in the case of sexual differences in chemical prey preferences, what we may see is actually an interaction of sometimes conflicting patterns of adaptive preference.

The moderate aggregative tendencies of this species somewhat explains the relatively weak response to conspecific feces cues in site choice testing. Where it occurred, preferences did seem to be in favor of the familiar diet, regardless of litter. This may imply a greater import of familiarity than the dietary cues themselves. Responses were simply too weak to compare the preferences of animals based on housing group, since the responses of animals in mixed-diet groups were the key to deciphering this particular riddle. This may be directly related to the association with familiar animals in aggregation.

A comparison of the results found and thus far discussed within T. sirtalis would now benefit from a comparison with results obtained with other species in similar experiments. As previously described, similar work has been performed using both T. radix and T. butleri (the latter under nearly identical conditions as those utilized for T. sirtalis here). Some of the results obtained in these two species differ greatly from those reported here for T. sirtalis, and are worth some discussion.

Comparison with Other Species

Comparisons with results of previous work have been made regarding each experiment in this dissertation. Table 7.3 facilitates this endeavor by comparing the major results of each experiment between the T. sirtalis subjects of the current research with those of the T. butleri previously studied within the same research design (Lyman, 1990).

The high initial response shown by T. sirtalis to chemical cues of both prey was more similar to another dietary generalist, T. radix (Burghardt, 1990), than to the specialist T. butleri, that only responded to chemical cues of its natural prey, earthworm (Lyman, 1990). Thamnophis butleri also did not show any differences in

Table 7.3: Comparison of major results of different experiments between the dietary generalist Thamnophis sirtalis and the worm specialist Thamnophis butleri in the same research design.

Dependent Variable	Independent Variable	T. butleri	T. sirtalis
Growth	Diet:	no effect	no effect
	Litter:	no effect	Litter 16 larger than litter 10
	Sex:	Males larger than females	no effect
	Group:	Less increase housed with T. sirtalis	no effect
	Overall:	More growth between 1st & 2nd reading	More growth between 2nd & 3rd reading
Initial Chemical Prey Preference	Diet:	no effect	no effect
	Litter:	no effect	Litter 10 strong worm, Litter 16 no preference
	Sex:	no effect	no effect
	Group:	Highest response housed with T. sirtalis	no effect
	Overall:	Strong worm preference	Weak worm preference
Final Chemical Prey Preference	Diet:	Preference for familiar prey, especially in worm fed	Very weak preference for familiar prey cues

Table 7.3: Continued.

Dependent Variable	Independent Variable	*T. butleri*	*T. sirtalis*
	Litter:	no effect	Litter 10 strong worm preference, litter 16 weak fish preference
	Sex:	no effect	no effect
	Group:	Isolates with low responses	Isolates with low responses
	Overall:	Worm preferred	Weak worm preference
Initial Feces-based Site Choice	Diet:	no effect	Fish diet prefer worm
	Litter:	no effect	Litter 10 prefers worm
	Sex:	no effect	Males prefer worm
	Group:	no effect	no effect
	Overall:	Prefer worm	Prefer worm
Final Feces-based Site Choice	Diet:	Worm-fed prefer fish	no effect
	Litter:	no effect	no effect
	Sex:	no effect	Males prefer worm, females prefer fish
	Group:	no effect	no effect
	Overall:	Weak fish preference	Weak worm preference

Table 7.3: Continued.

Dependent Variable	Independent Variable	T. butleri	T. sirtalis
Prey Chemical Site Choice	Diet:	no effect	Worm-fed prefer worm
	Litter:	no effect	Litter 10 prefer worm
	Sex:	no effect	Males prefer worm
	Group:	no effect	no effect
	Overall:	No preference	Prefer worm
Aggregation	Diet:	Both prefer opposites	Both nearer worm-fed
	Litter:	Prefer siblings	Litter 16 prefer sibs
	Sex:	no effect	Females prefer same sex, males prefer opposite
	Group:	Prefer Unfamiliar	Prefer Familiar
	Overall:	Few, large groups	Scattered, small groups

Note: Data for T. butleri from Lyman, 1990.

prey preference between animals in different litters, as the T. sirtalis did.

After experience with prey, T. sirtalis showed more frequent shifts in chemical prey preference toward familiar prey, though less strongly than was seen in either T. butleri or T. radix. This may be due to a greater generalism in acceptance of prey of T. sirtalis than the other species investigated (Carpenter, 1952a; Fitch, 1965). Again, strong differences were seen between animals in different litters only among the T. sirtalis. There was also an interesting pattern in which T. sirtalis housed with animals on either diet did not routinely show preferences for their own familiar diet, as was the case in single-diet housing groups. Among T. butleri there was no such effect of housing group on prey preference. This may indicate some social effect upon prey preference among the T. sirtalis.

In site choice testing, T. sirtalis showed an initial preference for sites marked with feces of conspecifics fed worms, corresponding with an overall initial preference for worm prey cues that was similar to that seen in T. butleri. However, after experience T. sirtalis preferred sites marked with feces of animals fed the familiar prey (if at all), while both T. butleri and T. radix had shown preferences for the opposite type of feces (Burghardt, 1990; Lyman, 1990). In this test, there was also an

effect of litter found only in T. sirtalis such that males more often preferred worm-based feces cues, whereas females more likely preferred fish-based cues. Regarding site choice based on prey surface cues, T. sirtalis responded similarly to dietary generalist T. radix, both preferring sites marked with cues of the preferred prey, whereas worm specialist T. butleri showed no preferences.

These differences between the responses of the species continue into aggregation testing. Thamnophis butleri had shown a significant preference to be near unfamiliar animals, while T. sirtalis often showed a preference for familiar animals. It was noteworthy that this tendency was strongest in animals housed with snakes on the opposite diet, indicating that familiarity was not confounded with diet in this case. Diet plays a generally weaker role in aggregative patterns in T. sirtalis than T. butleri, although snakes can apparently discriminate between others on different diets.

The preference for littermates observed in T. butleri is repeated in T. sirtalis, especially in one litter, indicating that kin recognition is possible in snake aggregations. The implications of this are discussed at greater length in chapter 6. Thamnophis sirtalis also demonstrated an effect of sex in aggregation preferences not seen in T. butleri which corresponds with aggregation

patterns of adult snakes of different sexes (e.g., Crews and Garstka, 1982; Gregory, 1975).

There were also general differences in activity and aggregation between the species, in that T. sirtalis were more active than T. butleri, and showed weaker site fidelity and preferences for specific shelters. However, animals from this population of T. sirtalis may be highly active compared to other populations of the same species (Schwartz, 1989). Thamnophis sirtalis were sometimes even observed resting in the open, a behavior never seen in T. butleri, which was in general a more secretive and tightly aggregating species (Lyman, 1990).

Regarding the interactions between tests, there are also some species contrasts. It was found that initially smaller T. butleri tended to have higher responses to the unusual fish prey cues. Feeding upon fish may confer enhanced growth due to increased calcium intake, though there is no evidence of T. butleri actually utilizing this prey in the wild. However, smaller T. sirtalis were found to show higher responses to worm cues. This seems to be related to the higher worm responsiveness of the smaller litter 10 animals, and possibly reflects a preference for a more ubiquitous prey.

In association measures, T. butleri in close associations tended to be larger animals. This is also true in general for T. sirtalis, probably due to the

correspondence of these factors in litter 16 snakes. However, in litter 10 animals it was the initially smaller snakes that were stronger aggregators, these individuals also being strong responders to worm chemical cues.

The greatest difference between T. sirtalis and the other species used in this experimental regime therefore seems to lie in the different utilization of conspecific cues, corresponding with a preference for familiar rather than unfamiliar individuals. Patterns seen in one species of garter snake therefore cannot be assumed true of other species, regardless of ecological similarity.

Social Aggregation of Garter Snakes

In the introduction, the definition of the term "aggregation" and its possible social implications were discussed. For this dissertation it was decided to use the term solely in a descriptive manner. At this point, however, we should return to this question. Aggregation in snakes has long been described, but has been generally explained regarding access to resources or favorable habitats (Allee, 1938; Immelmann and Beer, 1989; Scott, 1956).

In the laboratory experiments reported in this work it has been demonstrated that when juvenile T. sirtalis are attracted to conspecifics and their cues, that attraction must be based on characteristics of other individual

animals, since environmental factors have been controlled. This implies a non-random basis to this behavior: sites were chosen based on the occurrence there of other snakes with particular characteristics regarding litter, sex, familiarity, and sometimes diet. Such results establish the plausibility of information extraction and/or kin recognition in these reptiles. If the attraction were solely for improved environmental conditions afforded by clustered animals, there should have been no such patterns seen among the animals. That characteristics of particular animals is important in aggregation seems to demonstrate that such snake aggregations may be considered social behaviors.

Further Research

These experiments have also shown that much is still in need of clarification. There is a need to further refine investigations of the interaction of familiarity, kinship, and diet in aggregation behavior. Experiments must be performed using situations in which these factors are even more stringently controlled and manipulated than in previous studies. A larger number of both familiar and unfamiliar individuals available on each diet would be requisite for such research, as well as a greater number of litters to be compared across. Determination of the heritability of the behaviors in question would also be

useful, as would be comparisons across generations of animals with different preferences.

The possibility of kin recognition in garter snakes suggests an entire line of research into this phenomenon, both independent and interactive with effects of familiarity and factors such as diet. This needs to be extended into mate preferences of garter snakes relative to kinship as has been investigated in other species such as mice and Japanese quail.

The information that can be carried in integumental cues should also be investigated. Earlier work (Graves and Halpern, 1988) demonstrated attraction of garter snakes to conspecific skin extracts, and more recent findings continue to support this (Graves, Halpern and Friesen, 1991). What kind of information can be carried in such integumentary cues? Can these cues carry the same types of information as feces? The site choice tests used in this work would prove useful here, using integumentary cues from animals on different diets, of each sex, of differing litters, even familiar and unfamiliar individuals, as well as different species and/or populations.

Perhaps most importantly, patterns of aggregation need to be determined regarding individual movements and interactions. Do specific animals settle in a site first and attract others? If so, what are the characteristics

of these animals? What is the pattern of site choice; do snakes find a site and stay there, or do they investigate several alternatives first, and if so, what are the deciding factors? Similarly, the pattern of defecation of snakes around aggregation sites needs to be determined to find how animals may be utilizing this information in nature. Repeated observations of groups of snakes and their interactions must be made, possibly facilitated by filming observations to record movement patterns of snakes over time.

It is also clear that different species of snake, even within the same genus, may have very different patterns of behavior regarding aggregation and its underlying mechanisms. This research should continue to be extended into other species of garter snake. This will allow comparative studies of patterns in aggregation behavior related to both ecology and phylogeny of the species.

Overall, at least as many questions have been raised by the research described here as have been answered. Thamnophis sirtalis do show effects of dietary experience on prey preferences, which affect site choice based on prey cues. These snakes choose sites based on conspecific feces cues that may carry information regarding familiarity as well as dietary cues. Thamnophis sirtalis juveniles also show aggregative behaviors with patterns of individual characteristics, which implies social factors

in attraction and not just habitat choice based solely on environmental conditions. Most intriguing is the possibility that these snakes employ kin recognition, a phenomenon not previously reported in snake aggregation. The nature of attractants in these aggregations is still uncertain, as are possible dynamics among individuals and their relationship to the natural environment of this, and other, species of garter snake.

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VITA

Lani Patricia Lyman-Henley was born May 1, 1964, in San Francisco, California, and was raised by her parents, Harry Patrick and Violet Janet Kafka Lyman, and their two dogs, Butch and Mike. She caught her first snake at the age of 3 1/2 years, and devoted the next 10 years to convincing her mother to let her have one as a pet. This endeavor resulted in a genuine love of snakes and learning about them.

While a junior in high school working on a science fair project (Genetics in Garter Snakes), Lani met Dr. Harry Greene. This fateful meeting turned her toward learning as a profession, resulting in a Bachelor of Arts degree in Zoology at the University of California at Berkeley in 1987. She then decided to continue her studies with Dr. Greene's own mentor, Dr. Gordon Burghardt, at the University of Tennessee, where she completed a M. S. degree in Life Sciences: Ethology in 1990, followed by the degree of Doctor of Philosophy in Life Sciences: Ethology in 1991.

On December 21, 1990, Lani married Dr. Tracy Branson Henley. Both are avid collectors of various things (Lani's specialties mostly animate, Tracy's inanimate), between them accumulating a heck of a lot of stuff very quickly. This became acutely noticeable when they loaded down a very large truck in order to move to Starkville,

MS, where Tracy is currently an Assistant Professor in the Psychology Department at Mississippi State University.

Lani Lyman-Henley is actively planning research and pursuing grants both in collaboration with MSU faculty and independently, and is looking forward to instructing classes in the MSU Biology Department as early as Fall, 1991. She also looks forward to resuming her neglected artistic pursuits, breeding snakes of interesting colors, relaxing, and keeping her collections competitive with her husband's.