To the Graduate Council:

I am submitting herewith a thesis written by Lani P. Lyman entitled "The Effects of Dietary and Social Experience on the Development of Behavior in Butler's Garter Snake, Thamnophis butleri." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Life Sciences.

Gordon M. Burghardt

Gordon M. Burghardt, Major Professor

We have read this thesis
and recommend its acceptance:

John C. Eichenseht

T. C. Malone

Accepted for the Council:

Vice Provost
and Dean of The Graduate School

THE EFFECTS OF DIETARY AND SOCIAL EXPERIENCE ON THE DEVELOPMENT OF BEHAVIOR IN BUTLER'S GARTER SNAKE, THAMNOPHIS BUTLERI

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Lani P. Lyman

August 1990

ACKNOWLEDGMENTS

I wish to gratefully acknowldedge the many people who have aided in the completion of this thesis in various ways.

First I should like to thank the members of my committee. Dr. Gordon M. Burghardt, my major professor, provided me with guidance, encouragement, and support in my endeavors. He introduced me to snake social behavior, and his enthusiasm for learning is contagious. I am also quite impressed with his ability to read large numbers of verbose drafts quickly and seriously. I thank Dr. Arthur Echternacht for his exhaustive editorial review of this manuscript; his many comments and suggestions were of great help to me. Dr. John Malone also contributed greatly to the final revisions of the thesis, provided entertaining disscussions, and reminded me that not everyone knows "snake jargon." Although not a committee member, Dr. Mark Miller made very helpful suggestions regarding the analyses of aggregation used in chapter 6.

I also thank my colleagues and friends, Bonnie Bowers, Paul Andreadis, Dr. Cary Yeager, Mark Milostan, and Paul Doughty, and for help in setting up for experiments, feeding snakes in a pinch, providing editorial comments, and/or just good conversation over lunch and caffeine breaks. Especial thanks go to Donna Layne, for taking

care of many of my animals whether she had the time or not, her knowledge and consideration of snake husbandry and well-being, and for helping me glue many little pieces of paper with snake poop on them into test boxes.

Also deserving thanks are the many undergraduates who have assisted me in the course of this work. Among them, Robin McCallie and Jack Scheer helped greatly with computer data entry. Linda Dail, Stephanie Anderson and Chuck Boothe went above and beyond the call of duty in caring for nearly 200 young garter snakes (and thereby freeing me to write).

The influence of some very special people back home cannot be forgotten. A very special thank you goes to my parents, for their continuing support and encouragement, despite the snakes in the guest room and fish in the freezer. I would have never made it this far without them. Also a thank you to Dr. Harry Greene, who convinced me that I wanted to study snakes with him, and later continue my studies here with Gordon Burghardt.

And finally, I would like to extend heartfelt thanks to Dr. Tracy Henley, whose never-ending encouragement, support, and patience have brought me through some trying times. He has also provided editorial comments, aid with computer graphics, his organizational skills, and vast quantities of time, be it for work or play.

ABSTRACT

One way in which animals not thought to have complex social interactions can be viewed as nevertheless engaging in truly social behavior is through information transfer. A series of studies were designed to explore and develop this possibility in neonate Butler's garter snakes, Thamnophis butleri (Colubridae).

Thirty-eight T. butleri neonates from 2 litters of a Michigan population were measured, then tested prior to feeding for initial chemical prey preference 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 preference, and fed either worms (Lumbricus terrestris) or mosquito fish (Gambusia affinis). Two additional groups consisted of 4 T. butleri housed with 4 T. sirtalis on the same diet. The remaining 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.

The main differences in mass and snout-vent length were between the sexes, with males being both heavier and

longer than females throughout the study. One litter showed greater increases in mass, the other in length. Snakes also showed a general "slimming" of body shape over the course of the observations. The lack of difference in growth between diets should translate into a negligible difference between health of the subjects.

The T. butleri in this experiment 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 fed worms responded much more strongly to worm than fish cues, while snakes fed fish showed high responses to both prey, slightly favoring fish.

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 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 greater distances separating them from their nearest neighbors. There was also some indication that initially smaller snakes showed higher responsiveness to fish cues than larger animals.

It is hypothesized that familiarity is the most important factor in aggregative behavior, with dietary experience being secondary. The interactions of dietary experience with the feeding specialization of T. butleri, and possibly ancestral chemical preferences are also discussed. Further research into the cues involved in aggregative behavior is suggested.

Table of Contents

List of Tables

List of Figures

Figure Page

CHAPTER 1

INTRODUCTION

Aggregative Behavior

The phenomenon of animal aggregation ranges from such clearly and highly social species as those in the Orders Hymenoptera and Isoptera to species that appear to have no meaningful social interactions at all. The term "aggregation" is used regarding a diversity of animal grouping behaviors, but it is not always clear whether any sociality is inferred by it.

Scott (1956) suggests that for many animals most aggregations are dependent primarily on environmental conditions and are therefore irregular, nonspecific and not truly social. Allee (1938), a pioneer in the study of animal aggregation, describes even mating aggregations in some species as merely a simple crowding of nonsocial animals, such as snakes. More recently, 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. They refer to it as "subsocial" grouping, since it is on the boundary of "true" social grouping behaviors.

A variety of definitions of such "true" social behavior exist (e.g., Emlen, 1980; Tinbergen, 1953;

Wilson, 1975). One purpose of such definitional considerations is to distinguish evolutionarily meaningful social behaviors from mere accidental aggregation. Among defining factors of social grouping 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 or part of life cycle in which the group remains together. A third consideration that has been suggested is the minimal amount of time or energy spent in social behavior. A fourth concern requires reciprocal communication between group members. 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 (e.g., Wilson, 1975).

Following Burghardt (1983), the term "aggregation" will be used here in a purely descriptive sense without allusion to determinants. Indeed, one major purpose of the present study will be to better develop our understanding of aggregations by utilizing the snake Thamnophis butleri (Colubridae).

Functions of Grouping Behaviors

Tinbergen (1953) discusses examples of several benefits of social group living. Defense against predators, one such benefit, was exemplified by the tendency for birds to prey upon caterpillars leaving a

group, but not on the group itself. Likewise, this is seen in goldfish that have difficulty feeding upon clumped Daphnia due to the fish's tendency to switch targets before completing a capture. Goldfish also show increased growth, eating more and gaining more weight relative to food ingested, when in groups than when alone. Goldfish and Daphnia in groups both showed an increased tolerance to adverse environmental conditions such as metal toxicity and hypersalinity. Social facilitation of learning in cockroaches was also discussed. When allowed to run mazes in pairs or triplets, cockroaches learned faster than when trained individually. Note that none of these examples are taken from endothermic vertebrates such as birds and mammals, or eusocial insects such as ants and bees, that studies of social behavior usually emphasize.

Benefits of group living in addition to defense against predators 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 (Wilson, 1975).

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. Take for example the dramatic aggregations of snakes prior to

hibernation, wherein hundreds of snakes may converge within a few yards of an annual hibernaculum (e.g., Duvall, King and Gutzwiller, 1985). It can be argued in this case that the attractant is a common resource, however, what about the frequent aggregations of snakes outside of the hibernation or reproductive season that do not show effects of environment (Barbour, 1982; Dundee and Miller, 1968)?

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, which are generally considered nonsocial but do aggregate (Allee, 1931, 1938; Brattstrom, 1974; Wilson, 1975).

Typically what distinguishes a meaningful social behavior from a "mere aggregation" has been the social functions outlined by Wilson (1975). 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 which 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 aggregations without meeting such aforementioned specific criteria.

Aggregation in Reptiles

Reptiles have been considered to lack complexity in many aspects of behavior (Brattstrom, 1974), and snakes have been described as the least social of reptiles (Allee, 1931, 1938; Brattstrom, 1974; Prater, 1933; Wilson 1975). The former view is mistaken and the latter exaggerated. Reptiles are dynamic and active animals when their environmental constraints, such as optimal temperature, are taken into consideration and when relevant stimuli, such as heat sources for diurnal lizards or hiding boxes for nocturnal ones, are used (Brattstrom, 1974; Burghardt, 1977; Gillingham, 1987). Brattstrom (1974), though considering snakes less social than other reptiles, admits that little is known of them and suspects they are more socially active than we currently believe.

Social behaviors seen in snakes include male combat, courtship and mating, including large mating aggregations (Carpenter, 1977; Crews and Garstka, 1982; Finneran, 1949). Aggregation for hibernation (Duvall, King and Gutzwiller, 1985; Noble and Clausen, 1936), and communal nesting (Swain and Smith, 1978) have also been documented. Dominance hierarchies have even been reported for male

Indian pythons, Python molurus (Boidae) (Barker, Murphy and Smith, 1979). Although the aggregations for purposes of reproduction and hibernation are well documented, other aggregations frequently occur for which the reasons are not always so clear. Noble and Clausen (1936) reported that 63% of brown snakes, Storeria dekayi (Colubridae), observed outside of hibernation were in aggregations (excluding gestating females, which did not aggregate).

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 in snakes (Gregory, 1975), as in many other grouping animals (e.g., Hamilton, 1971).

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) in a northern part of their range, where grouped snakes warmed more quickly and cooled more slowly than isolated snakes.

Enhanced foraging has been suggested in several studies. Kropach (1971) reports that sea snakes, Pelamis platurus (Hydrophiidae), in aggregations consistently had stomachs "packed with food," while those found away from these groups rarely contained prey items. Barbour (1962) described an aggregation of copperheads, Agkistrodon

contortrix (Viperidae), in which most of the snake's stomachs were full of cicada nymphs, although there was no particular abundance of these prey in the immediate vicinity. Schwartz and Allen (in Burghardt, in press) have demonstrated increased growth rate in group housed neonate water snakes, Nerodia sipedon (Colubridae). In the latter study, snakes raised in groups, but fed individually, averaged a greater weight gain than animals kept isolated or fed in groups. Group-fed animals showed competition for food to the detriment of some individuals.

All of this varied research suggests that the benefits of aggregation within snakes parallel those of meaningful social behaviors. Nevertheless, more systematic experimental research remains needed to advance our understanding of aggregations in snakes. Research on a variety of other species has uncovered a number of influences and ideas concerning aggregation that should be considered.

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. Galef (1986, 1988) subsequently demonstrated this phenomenon in rats, Rattus norvegicus. 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 demonstrator was anaesthetized or dead at the time of "interaction," and the information was found to be via olfaction. Subsequently, Porter, McFadyen-Ketchum and King (1989) reported similarly that spiny mice, Acomys caharinus, associated more often with other mice that had been fed the same natural or artificial diet.

Both kinship and familiarity have also been suggested as important factors related to aggregation. A preference for unfamiliar kin over familiar non-kin has been demonstrated in Japanese quail chicks, Coturnix c. japonica (Waldman and Bateson, 1989). Porter, Matochik and Makin (1986) demonstrated a similar kin preference in spiny mice that was accentuated by familiarity. White-footed mice, Peromyscus leucopus, preferred familiar animals regardless of genetic relationship (Halpin & Hoffman, 1987). Age and sex have also been shown to

influence grouping behavior in 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).

The situations described above seem to constitute a case in which actions that are typically thought of as nonsocial can nevertheless transfer biologically significant information. Perhaps then, animals such as snakes, engaging in what would otherwise appear to be a "nonsocial" aggregation, may be engaging in such "information transfer."

Before determining whether snakes could be engaging in something like information transfer concerning diet, kinship or familiarity, we require an understanding of what sensory mechanisms snakes may be using in the reception of such "social" information related to aggregative behavior.

Sensory Control of Snake Behavior

Aggregation and Habitat Choice

Noble and Clausen (1936) investigated the role of the senses in aggregation behavior of the brown snake, Storeria dekayi. Vision was eliminated by adhesive tape or blackened collodion placed over the snake's eyes. Olfaction was eliminated by plugging the nostrils with cotton, vaseline, and collodion. The tongue was severed

just caudal to the bifurcation to inhibit chemoreception, and in some cases the vomeronasal organ (VNO) was cauterized. Nose-stopped animals did not aggregate in the dark, and if also blindfolded did not aggregate in the light either. Tongueless snakes aggregated almost as well as controls. From these results they concluded that vision, and less extensively olfaction, were the primary senses used in aggregation and that the vomeronasal organ played little or no part. In a comparison using the same sense-deprived snakes to follow earthworm-scented trails, it was again the nose-stopped animals that showed the greatest handicap.

In contrast, Wilde's (1938) carefully controlled work in which the vomeronasal nerves were severed demonstrated 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). Indeed, Burghardt and Pruitt's (1975) work demonstrated that snakes could still show VNO functioning with any protrusible stub of tongue, and that complete tongue removal generally stopped feeding behavior. Noble and Clausen (1936) stated that only animals that had resumed eating were used in their experiments. This coupled with the findings of Burghardt and Pruitt (1975), and Wilde (1938), would suggest that

the vomeronasal organ was still functioning in their test animals.

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 that 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).

Heller and Halpern (1982a) demonstrated that the relevant cues for locating favored sites in common garter snakes, T. sirtalis, are deposited on the substrate. Heller and Halpern (1982b) 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." This information is interesting to compare to the findings of Porter, Sentell, and Makin (1987) that anosmic spiny mice raised with intact animals did not show the same abnormal behaviors as those raised only with other anosmic pups.

The identity of the exact chemical stimulus for aggregation behavior is still uncertain. Graves and Halpern (1988) showed that neonate T. radix demonstrate preferences for shelters marked with chemical extracts of conspecific shed skins. 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). Several studies have since demonstrated that some snakes, including the plains garter snake, T. radix, and water snakes N. sipedon and N. cyclopion 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, that originally attract snakes to an area occupied by other snakes, it is possible that information transfer may occur via feces. Chemical cues related to diets such as fish can be detected in garter snake feces even by human olfaction (L. Lyman and D. Layne, personal observation), and it is entirely likely that snakes could discern far more subtle dietary cues in feces and possibly use that information in choosing habitat sites.

Burghardt (1983) used neonates 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 many innate responses to chemical cues of prey prior to experience (as will be discussed below), and that they do not need such cues to show aggregative behavior does not rule out that they may use such cues to modify this behavior once available. In this thesis the possibility that feces, containing information about diet

(that is, available prey), could be a basis for information extraction is considered. 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 a snake 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) investigated the scoring of tongue-flicks combined with the latency to attack, where high tongue-flick rate and short latencies indicating predatory interest in a stimulus, and developed the Tongue-Flick Attack Score (TFAS), which is the measure to be used here. Cooper and Burghardt (1990) compared this to several other measures of interest in chemical

prey cues and found TFAS to be the most useful. Related methodological considerations such as the most effective preparation of surface extracts of prey, and the nature of the substances eliciting prey attack behavior have also been investigated (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 common 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 or 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. couchi, 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 affect 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 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 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 or fish species used. Animals fed fish paralleled this pattern, with stronger responses to the familiar species of fish. This phenomenon of repeated exposure to a stimulus increasing attractiveness without reinforcement has been described by Zajonc (1971). The plains garter snake, T. radix, has been shown to exhibit such response shifts in chemical prey preference based on experience (Burghardt and Lyman, in preparation). Animals in the same litter raised on different diets of earthworm or fish shifted preference towards the experienced item regardless of initial preference. This species is a generalist feeder known to feed on both worms and fish in the wild.

Aggregative behavior of snakes, its possible functions and sensory components have been summarized above. The chemical senses have been demonstrated to play an important role in both aggregation and response to prey cues, the latter of which snakes show preferences towards which can be changed through experience.

From the review of the above studies a program of research is suggested that would consider such factors as kin and sex along with a focus on diet with in 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.

Experimental Rationale for the Present Study

In a previous experiment using 24 neonate plains garter snakes, Thamnophis radix, G. M. Burghardt and I investigated the correspondence of chemical prey preferences with habitat choice (summary in Burghardt, in press; Burghardt and Lyman, in preparation). 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. 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 habitat 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 habitat 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 than those fed fish.

This intriguing result implies that plains garter snakes are able to discriminate between conspecific feces on different diets and use this information to choose habitat sites. However, the attraction seems to be in favor of habitats marked by animals feeding on the non-preferred diet.

It seems possible that given their high degree of reliance on chemical cues, snakes could be using conspecific feces cues as an attractant for aggregation. It is also possible that they may be able to gather information regarding the diets of other animals in the area when choosing these habitat sites. Neonate snakes show immediate preferences for certain prey which can be modified through experience. Could these prey preferences be reflected in aggregative behavior? What about other possible attractants seen in other species, such as relatedness or familiarity?

To investigate these questions, an experimental regime was designed in which neonatal garter snakes would be raised in group housing situations on diets of fish or earthworm and tested for prey preference, site choice based on prey and conspecific cues, and finally, in arena aggregation. In this way the effects of diet, prey preference, familiarity, litter, and sex could all be evaluated in habitat choice and aggregative behavior. Concurrently, body length and mass measurements would be recorded in order to track growth rate of animals by each variable. Rates of growth could be taken as an indicator of general health, and be used to determine the equivalence of the two diets in maintenance of the subjects.

The animals used were Butler's garter snakes, Thamnophis butleri, from 2 litters originating in 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, in press; Burghardt and Lyman, in preparation), and its tendency to aggregate (Carpenter, 1952a; 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.

Based on the idea of information transfer and previous ecological studies of this animal, the following hypotheses were formulated:

1. Although T. butleri will respond naively to both worm and fish extracts, the initial response is expected to be stronger to the earthworms since they form the bulk of the species typical diet.

2. Since this species is an earthworm specialist, the responses to chemical prey cues after experience with prey should also favor earthworms, though the animals fed fish should show a higher response to that prey than inexperienced snakes.

3. Site choice should be in favor of the preferred prey cues: earthworm. This would make ecological sense, since prey should be more likely to be found where its chemical cues are strongest.

4. Site choice based on feces should also be in favor of cues based on the preferred prey. If the animals prefer to be in association with other snakes feeding on the same preferred prey, then perhaps the reason is to find areas rich in that resource.

5. These snakes should aggregate preferentially with other snakes feeding on the same, preferred, diet. This would indicate the use of information transfer via feces

cues to locate areas rich in preferred prey based on information gained from other snakes in the area.

A report of results from the execution of this program of research is what follows in the remainder of this thesis. Chapter 2 will discuss general methodology (e.g., origin of animals, housing, feeding). Chapter 3 presents data on growth in animals on different diets. These data, as mentioned above, are important to establishing equivalent health of animals on different diets, and as such are essential to a design that would compare across diets. Chapter 4 details how dietary experience affects prey item preference as measured through standard swab testing. Chapter 5 examines habitat 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.

CHAPTER 2

GENERAL METHODOLOGY

Overview

This chapter details the origin, identification, housing and diet of the subject animals, Thamnophis butleri, used in this study. Particular experimental methods are presented within the chapter detailing individual studies.

Species Description

Butler's garter snake, Thamnophis butleri, is found in Ohio, extreme southern Ontario, eastern Michigan, central Indiana, and a relic population exists in southeast Wisconsin (Conant, 1975). Ruthven (1908) and Carpenter (1952a) describe the microhabitat of this species in southern Michigan as being thick grasses and sedges along marsh margins and stream banks. Ruthven (1908) further comments that this description is consistent with data from the rest of the animal's range.

This species of garter snake feeds almost exclusively on earthworms and leeches in the wild (Carpenter, 1952a; Ruthven, 1908). Despite the absence of fish in the diet of wild snakes, T. butleri have been reported to accept fish in captivity (Halloy, 1987; Ruthven, 1908).

Thamnophis butleri is closely related to T. brachystoma, T. radix, T. marcianus, and T. eques (previously: T. megalops) (Ruthven, 1908). It is considered to have derived directly from T. radix (Conant, 1950; Davis, 1932; Schmidt, 1938).

Subjects

Subjects for the studies to follow were derived from two litters of Thamnophis butleri. Gravid females were collected in Wayne County, Michigan, by a commercial dealer and arrived at the University of Tennessee at Knoxville on June 2, 1988. Female T. butleri were housed at an ambient temperature of 22±2°C with a heat gradient to 28°C afforded by a heat tape under one side of their containment tank. Neonates were born on July 3, 1988 to two females lab coded 88-MI-1239 (N=21) and 88-MI-1241 (N=21). Litters will be referred to from here on only by the last two digits of this lab code, which reflects maternal identity.

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 towel substrate. Animals were removed one at a time, in no specific order, and assigned a subject letter. Animals were weighed and measured (see also Chapter 3), and placed in individual 13 x 18 x 5 cm clear plastic containers. Each container contained substrate and folded "tent" shelter of Aniboard©

(a green paper board treated with neomycin to control odor) and a glass water dish. All snakes in containers were visually isolated from one another. Animals were kept at 20±2°C on a 12:12, light:dark, cycle.

The neonatal T. butleri were sexed by hemipenal eversion at 8 days of age by G.M. Burghardt, and were rechecked at 24 days of age.

Dietary Regimes

Neonates were placed into one of two dietary regimes; either fish (Gambusia affinis) or earthworm (Lumbricus terrestris). Every attempt was made to keep snakes naive to one of the two prey types. However, several snakes required repeated exposure to one or both prey items before one was accepted and a dietary group could be assigned. Getting neonates to accept fish was more difficult than getting them to accept worms. This is consistent with their reputation for difficult captive maintenance and the lack of fish in their natural diet.

Food was first offered at 12 days of age, and offerings were made twice daily. Fish were offered first to several snakes, balanced for litter, sex, size, and Initial Chemical Prey Preference. Another such group of snakes were offered worms, and a third set were offered one of each prey in alternating order. This process continued until all animals had accepted one of the prey

types on two consecutive offerings, and they were from then on fed only that prey. This method was used due to the expected high rate of refusal towards fish. Worms, once offered, are generally accepted promptly. A final grouping of half of the animals per diet was achieved after about six days. See Table 2.1 for composition of diet groups, and individuals exposed to opposite prey. Only four animals actually ate both prey types, and all of these were assigned to the fish diet to keep numbers balanced.

Prey animals

Mosquito fish, Gambusia affinis, were collected from wild populations using seine and hand nets. Numbers of fish taken per collection trip seemed to vary with temperature, water level, amount of algae on the water's surface, and with numbers of predatory centrarchid fish in the immediate area. Collected fish were maintained in the laboratory in two aquaria (120 and 40 liter capacity) and fed commercial tropical fish flake food until use. Captive propagation of these animals proved to be unfeasable.

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 early in the experiments, to insure acceptance. Additional movement and odor cues afforded in this manner of feeding generally stimulates the animal to strike quickly (Kauffeld, 1953). 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 25 mm, with an estimated mean of 13 mm. One to four, 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.

Table 2.1: Composition of groups of Thamnophis butleri (N=38) detailing early exposure to prey items.

			SUBJECT	
GROUP	DIET	LITTER	MALE	FEMALE
7	FISH	39	A*, N	F, K
		41	P, Q*	I**, L
8	WORM	39	G, P*	E, R
		41	N, R*	A, U
9	FISH	39	T*	S*
		41	C	M
	WORM	39	M*	Q
		41	S*	E
(mixed species with T. sirtalis)				
10	FISH	39	J*	I
		41	F**	O**
11	WORM	39	D*	C*
		41	J, B	
(individually housed animals)				
"12"	FISH	39	B*	O*
		41	-	D**
	WORM	39	-	-
		41	G*,H*,T	-

NOTE: "*" denotes animals exposed to both prey items (N=15); "**" denotes animals that had eaten both prey items (N=4).

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 as previous research (detailed in Chapter 3) 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 five housing groups, each group containing eight snakes, equated for mass and balanced for sex, litter, and initial chemical prey preference (described in Chapter 4). One group was made up of T. butleri on fish diets, another contained animals on worm diets, and a third consisted of four animals from each diet regime. The last two groups contained four snakes each of two species. One of these groups was on a worm diet, the other on a fish diet. The second species used was the common garter snake, Thamnophis sirtalis, originating from two litters collected from the same area in Michigan as the T. butleri, that were six days older. These groups were used for a comparative study that will not be detailed in this thesis. Six of the remaining nine animals were maintained in isolation, and the last three

were removed from the experiment due to a consistent refusal to accept either offered prey. Housing group compositions are detailed in Table 2.1 (page 28).

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 butleri were marked at 29 to 30 days of age. Snakes were held firmly under a dissecting microscope and scutes were counted forward from the vent. A combination of two scutes reflecting litter and subject codes were clipped on each snake with microdissecting scissors. Clips were made on the left side of the scute, and swabbed with isopropyl alcohol before each snake was returned to its tank. A marked scute located one anterior to the vent denoted animals of litter 39; clipping scute five, litter 41. Scutes from position 11 on forward corresponded with the alphabetical subject code of each snake. This is detailed in Figure 2.1.

At this time, animals were also observed for any naturally occurring anomalies along the posterior ventral

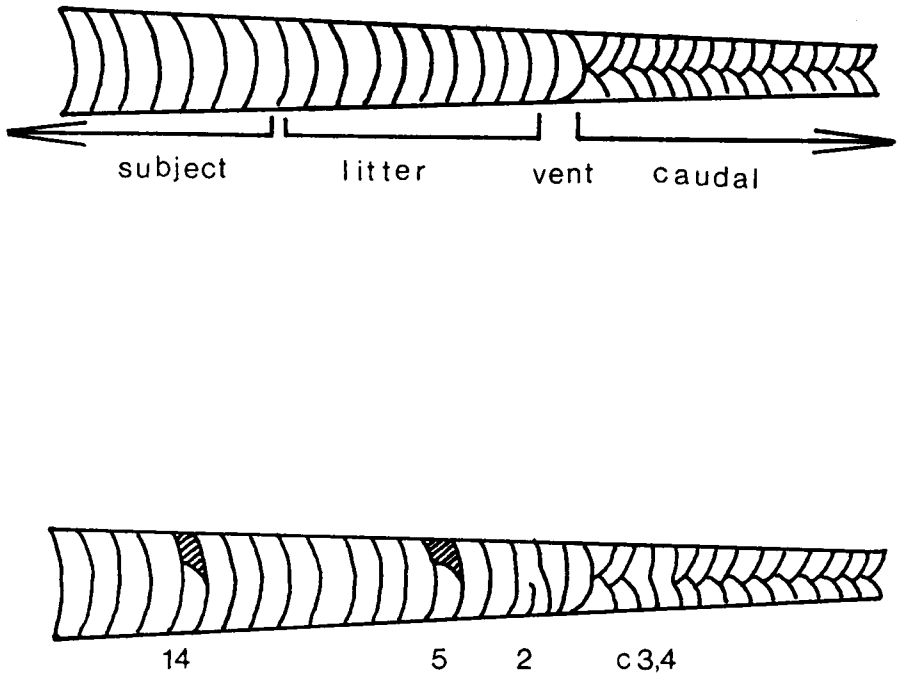

Figure 2.1: Ventral scale-clipping system for permanent identification of snakes.

NOTE: the top portion is a ventral view, the bottom portion is an example of a marked snake. This animal would be recorded as subject D (scute 14) from litter 41 (scute 5). Additional anomalies are that caudals number 3 and 4 are single, and ventral number 2 is doubled. and anterior caudal scutes, as well as of the labials. Such anomalies, often in unique combinations, can be of great help in identifying animals after clipping scars have faded or whose codes are very similar.

Snakes were also individually labelled for quick identification with head tags made from a light blue plastic tape. Litter 39 was labeled with triangular, and litter 41 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 butleri were placed in groups when 31 days old. Mixed species groups were formed at 38 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. 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 July 3. Initial measurements and tests were performed prior to the first food offering at 12 days, and final testing was performed beginning at 122 days of age. These experiments were completed when snakes

were 213 days old. The testing schedule is detailed in Table 2.2.

Subject Mortality

Between 31 and 85 days of age 3 animals spontaneously died, explaining the change in sample size between the initial and final Chemical and Site Choice tests. Cause of deaths was unknown, but occurred at different times and did not seem to be related. For the most part, statistical analyses utilize only the animals that lived throughout this period.

During the period from 160 to 218 days of age, another 8 animals died. One of these animals was another spontaneous death. However, 7 of the 8 animals died within a week of one another, and all were worm fed animals. These deaths are probably the result of diseased or toxic worms, Lumbricus, purchased a few days earlier. The worms in question did not look healthy, being relatively inactive, dark in color and somewhat "lumpy" or blistered in appearance. Due to a lack of previous experience with worms of this description, and also a lack of healthier looking worms to choose from at the time, they were still used. It is possible that the worms were diseased, parasite-infested, or accidentally fed something toxic before they had been purchased. The deaths of many other worm fed garter snakes of various species occurred concurrently with those in this study. Deaths occurred

Table 2.2: Testing Schedule for T. butleri used in thesis.

Days of Age	Test/Occurrence
1	First body measurements (N = 41).
8	Sexed (N=41).
10	Initial Chemical Prey Preference Test (N=41).
11	Initial Feces-Based Habitat Choice Test (N=41).
12	Prey first offered (N=41).
24	Resexed (N=41).
30	Placed into housing groups (N=41).
86	Second body measurements (N=38).
122	Prey Surface-Based Habitat Choice Test (N=38).
136	Final Feces-Based Habitat Choice Test (N=38).
159	Final Chemical Prey Preference Test (N=38).
219	Arena Aggregation Test (N=24).
231	Third body measurement (N=30), Experiments complete.

Note: Changes in N due to subject mortality except for Arena Aggregation Test, which did not include animals housed with T. sirtalis.

within a week of being fed the aforementioned worms and were accompanied by regurgitation and some intestinal blockages. The remaining ill Lumbricus were discarded and their appearance noted for future reference.

CHAPTER 3

GROWTH

Introduction

Growth curves in reptiles resemble step functions due to discontinuity as an effect of the external environment and shifts in allocation of energy (Andrews, 1982). Reptiles are capable of responding with high growth rates to increases in temporally available resources, then persisting during periods of low resource availability with quite low growth rates. 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, in press; Burghardt and Lyman, 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 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 the following experiment, the worm-specialist, T. butleri, closely related to the T. radix used in the experiments described above, 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 on were also analyzed.

Methods

Subjects

Subjects were 38 T. butleri during the first two observations and 30 during the last. In the last observation the total number of snakes was decreased by 8 due to subject mortality, as described in Chapter 2. Animals were derived from 2 litters (39 and 41) and maintenance is detailed in Chapter 2. Litter, sex, diet and housing information was 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 such that each regime was balanced as well as possible for sex, litter, size, and Initial Chemical Prey Preference (see Chapter 4). Exact diet assignment of individuals was constrained by acceptance of the offered prey as described in Chapter 2.

Mosquito fish, Gambusia affinis, ranged from 6 mm to 25 mm in total length. One to four fish were offered to snakes while still alive in water dishes. Fish meals were approximately 0.15 to 0.20 grams. Earthworms, Lumbricus

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

GROUP	DIET	LITTER	SUBJECT MALE	SUBJECT FEMALE
7	FISH	39	A, N	F, K
		41	P*, Q	I, L
8	WORM	39	G*, P	E, R
		41	N, R	A*, U
9	FISH	39	T	S
		41	C	M
	WORM	39	M*	Q*
		41	S	E*
(mixed species with T. sirtalis)				
10	FISH	39	J	I
		41	F*	O
11	WORM	39	D	C
		41	J, B*	
(individually housed animals)				
"12"	FISH	39	B	O
		41	-	D
	WORM	39	-	-
		41	G,H,T	-

Note: Group "12" are individually housed.

terrestris, were offered as freshly cut pieces ranging from 5 mm to 10 mm in length, and 2 mm to 4 mm in width. One to four pieces comprised a meal, weighing approximately 0.20 to 0.30 grams. Food was provided twice weekly to all subjects. Any prey items not eaten within 2 hours were offered to the snake on forceps, which generally stimulated acceptance.

Amounts of fish and worm were kept proportional, with a slightly greater mass of worm than fish being offered. 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, in press).

Toward the end of the observational period of the present study the quantities of available Gambusia decreased, necessitating a reduction in meal sizes. 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 here due to the high occurrence of "knob-tails" (curled, balled or otherwise shortened tail tips) in the subject animals, making tail lengths highly variable. The day-of-birth measurements were taken by B. Bowers and D. Layne.

All subjects were measured three times: on the day of birth, when the subjects were 85 days of age, and when 231 days of age. The first measurement was prior to any feeding. In order to minimize effects of gut contents, subsequent measurements were taken at least 3 days after snakes had eaten. Initial experiments occurred within 12 days after the first measurements, and all other experiments performed in the course of work for this thesis occurred between the 2nd and 3rd measurement observations. The testing schedule is detailed in Chapter 2.

Statistical Analyses

Values used for analysis were snout-vent length (SVL) and mass (MS). The difference of SVL and MS between each observation was also calculated (SVDIF and MSDIF) as a measure of absolute size increase. To achieve a relative growth value, the percent increase for SVL was calculated (PSV) using the formula:

$$SVDIF(b - a)/SVL(a)$$

where (a) is the SV length at the earlier observation and (b) is the later observation, and SVL(a) is the snout-vent length for observation a. The percent increase for MS (PMI) was calculated in the same manner.

Also calculated was the condition index (CI) of each animal per measurement time. This is a measure of volume which reflects body shape (e.g., "stout" or "slender"). It is described by the formula:

$$(MS/SVL^{**}3)100$$

where MS is measured in grams, SVL in cm. This value has been used in studies of common iguanas, Iguana iguana (Werner, Packard and Paton, unpublished), in which a higher value reflects a proportionately "fatter" animal than a low value.

Dependent measures SVL, MS, SVDIF, MSDIF, PSV, PMI, and CI, 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 using only the 30 animals that survived the entire period. Multiple range tests were utilized in tests involving groups or observations. Analyses were performed using Statgraphics 3.0 (STSC, 1988).

Results

Snout-Vent Length (SVL)

SVL refers to absolute length in millimeters. At the time of the initial observation of SVL at birth, there were significant differences between the two litters ($F(1,37)=5.93$, $p=0.022$) and sexes ($F(1,37)=5.36$, $p=0.028$). Litter 39 (mean SVL=124.8 mm) was longer than litter 41 (mean SVL=120.9 mm), and males (mean SVL=124.9 mm) were longer than females (mean SVL=120.1 mm). The animals that would later comprise the worm-fed group were nonsignificantly ($F(1,37)=3.46$, $p=0.074$) larger (mean SVL=124.2 mm) than those that would be fed fish (mean SVL=121.2 mm).

At the second observation, no differences were found among diet, litter, or group, although sex showed a nearly significant difference in SVL ($F(1,37)=4.04$, $p=0.055$), with males still longer (mean SVL=140.8 mm) than females (mean SVL=135.6 mm). At the third observation sex was again nearly significant ($F(1,29)=4.07$, $p=0.059$) with males longer (mean SVL=146.06 mm) than females (mean SVL=138.43 mm).

A repeated-measures ANOVA found a highly significant increase in length among observations ($F(2,89)=391.8$, $p<0.0001$). 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 122.3 mm, the second

mean SVL was 138.5 mm, and the final measurement averaged 142.5 mm, all three of which were significantly different from one another. Figure 3.1 summarizes the effects of diet, litter, and sex on SVL over observations. Table 3.2 is provided as a summary of the significant (and nearly so) findings across all tests utilizing discrete measurements taken per observation (SVL, MS, and CI).

Mass (MS)

This measurement refers to the absolute mass in grams of each snake per observation. At the time of the initial measurement, only sex showed a significant main effect on MS ($F(1,37)=7.05$, $p=0.013$), with males being heavier (mean MS=1.75 g) than females (mean MS=1.56 g). At the second observation sex was still the only variable significantly differing ($F(1,37)=5.17$, $p=0.031$). Males were still heavier (mean MS=1.99 g) than females (mean MS=1.81 g).

At the final measurement, there remained a significant difference between the sexes ($F(1,29)=6.02$, $p=0.025$) with males (mean MS=2.0 g) outweighing females (mean MS=1.71 g). Additionally, there was now a significant difference between the two diets ($F(1,29)=9.51$, $p=0.0064$) with animals fed worms weighing more (mean MS=2.00 g) than those fed fish (mean MS=1.76 g). Litter was not significant ($F(1,29)=4.03$, $p=0.059$), but litter 39 tended to be heavier (mean MS=1.874 g) than litter 41 (mean MS=1.856 g).

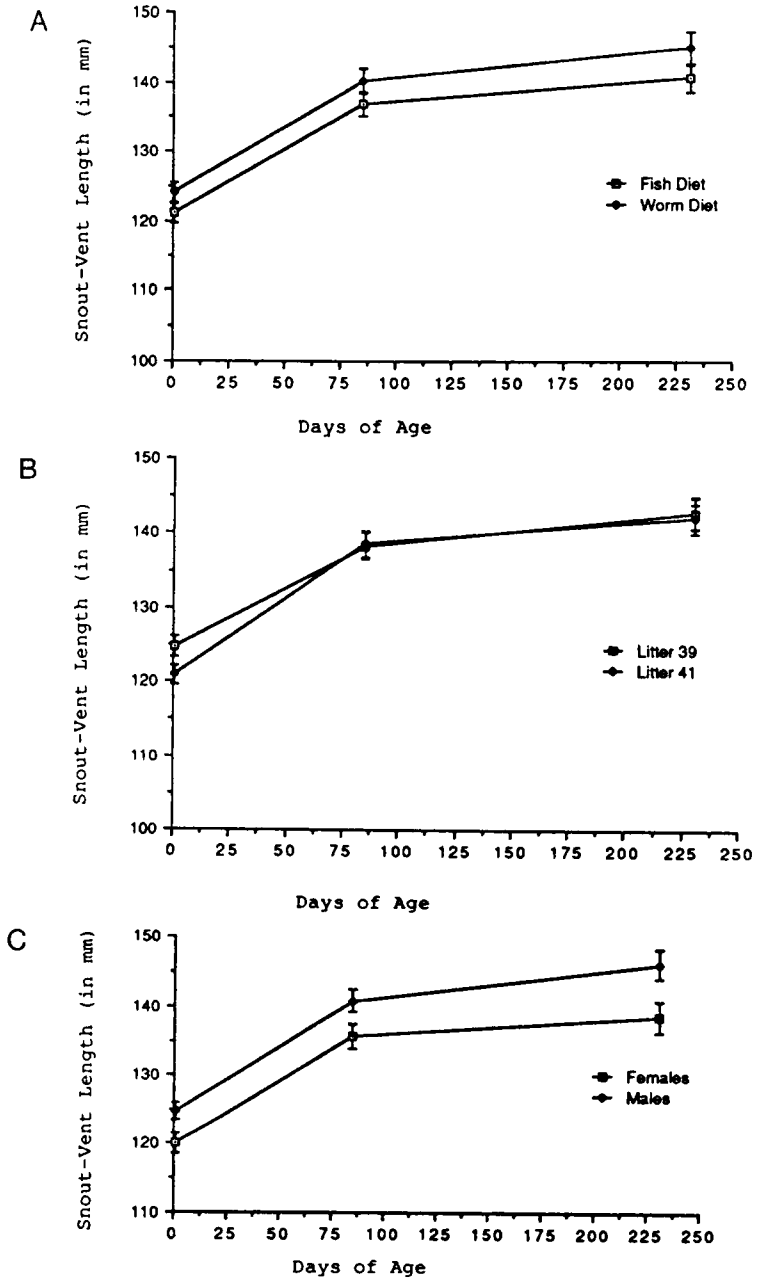

Figure 3.1. Snout-vent length (SVL) in mm ±1 standard error (SE) of T. butleri over time by A) diet, B) litter, and C) sex.

Table 3.2: Table of ANOVA p-values for discrete measures used in analysis of growth in T. butleri.

Variables	Measures SVL	MS	CI
Initial measurement			
Diet	.074	-	-
Litter	.022	-	.0007
Sex	.028	.013	-
Group	-	-	-
Second measurement			
Diet	-	-	-
Litter	-	-	-
Sex	.055	.031	-
Group	-	-	-
Interactions:			
diet x sex	-	-	.088
Third measurement			
Diet	-	.0064	-
Litter	-	.060	-
Sex	.059	.025	-
Group	-	-	-

Note: "SVL"=snout-vent length, "MS"=mass, "CI"=condition index. Values greater than p=.11 are denoted by a "-." All main effects are listed, interactions are listed only if occurring.

Predictably, mass increased significantly over the observations ($F(2,89)=37.90$, $p<0.0001$) initially averaging 1.64 g, having a mean MS at the second observation of 1.87 g, and at the final observation averaging 1.89 g. It should be noted that these weights were all very close, with only two tenths of a gram being a significant difference between observation 1 and the following two. Figure 3.2 summarizes the effects of diet, litter, and sex on mass over observations.

Snout-Vent Difference (SVDIF)

SVDIF refers to the difference between snout-vent measurements for each snake between observations, being an indication of growth in length. There were no significant effects on SVDIF between the first and last observations, although sex was nearly so ($F(1,29)=2.99$, $p=0.100$), with males showing a greater increase (mean SVDIF=21.9 mm) than females (mean SVDIF=18.4 mm).

However, a comparison of SVDIF between observations 1 and 2 and between 2 and 3 shows a somewhat different picture. Between observations 1 and 2 the only significant effect was litter ($F(1,37)=6.98$, $p=0.014$), where litter 41 increased more in SVL (mean SVDIF=17.75 mm) than litter 39 (mean SVDIF=13.56 mm). Between observation 2 and 3 the only significant effect was due to sex ($F(1,29)=4.58$, $p=0.046$) with males increasing more (mean SVDIF=5.63 mm) than females (mean SVDIF=3.14 mm). So the initial

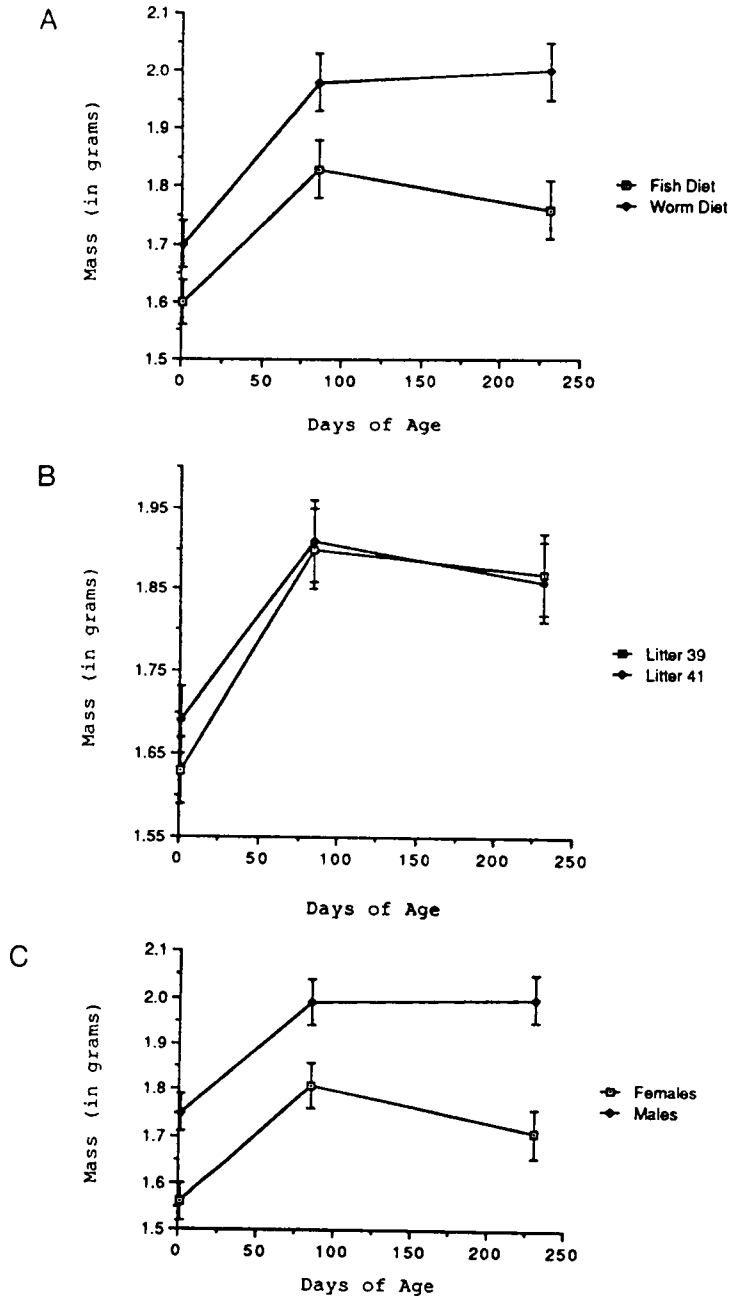

Figure 3.2. Mass (MS) in grams ±1 standard error (SE) of T. butleri over time by A) diet, B) litter, and C) sex.

difference between litters dropped out over time, while the difference between sexes increased.

There was a significant difference in SVDIF between observations ($F(2,89)=166.81$, $p<0.0001$). SVDIF from observations 1 to 2 averaged 15.73 mm, from 2 to 3 was 4.47 mm, and the overall growth from 1 to 3 averaged 20.20 mm, all of which differed from one another. The growth rate per day thus averages 0.182 mm over the first 85 days, and only 0.031 mm per day over the last 146 days of observation, with the average for the entire 231 day period being 0.087 mm per day. Table 3.3 summarizes these findings across all tests emphasizing changes between observations (SVDIF, MSDIF, PSV, PMI).

Mass Difference (MSDIF)

MSDIF refers to the difference in grams between observations of mass. It should give a measure of growth in mass. Between observations 1 and 3 the difference in litter is significant ($F(1,29)=6.58$, $p=0.019$) with litter 39 gaining an average of 0.288g while litter 41 gained an average of 0.199 g. There was a nonsignificant difference between diets ($F(1,29)=3.01$, $p=0.100$), with animals on the worm diet showing slightly greater mass increase (mean MSDIF=0.288 g) than those fed fish (mean MSDIF=0.209 g).

Between observation 1 and 2 the only significant effect on mass gain was housing group ($F(5,37)=2.93$, $p=0.032$). Group 11, worm-fed and housed with *T. sirtalis*,

Table 3.3: Table of ANOVA p-values for measures between observations used in analysis of growth of *T. butleri*.

Variables	Measures SVDIF	MSDIF	PSV	PMI
Observations 1 to 2				
Diet	-	-	-	-
Litter	.014	-	.007	-
Sex	-	-	-	-
Group	-	.032	-	.035
Observations 2 to 3				
Diet	-	.106	-	.049
Litter	-	.094	-	.026
Sex	.046	-	.067	-
Group	-	-	-	-
Interactions:				
diet x litter	-	-	-	.049
diet x sex	-	-	-	.012
Observations 1 to 3				
Diet	-	.100	-	.093
Litter	-	.019	-	-
Sex	.101	-	.039	-
Group	-	-	-	-
Interactions:				
diet x sex	-	-	.107	-
litter x sex	-	-	.047	-

Note: "SVDIF"=difference in snout-vent length, "MSDIF"=difference in mass, "PSV"=percent snout-vent increase, "PMI"=percent mass increase. Values greater than p=.11 are denoted by a "-."

showed the least mass gain (mean MSDIF=0.098 g) and group "12," those housed individually and half on each diet, showed the greatest gain (mean MSDIF=0.407 g). Multiple Range tests of the least significant difference (LSD) showed that group "12" (individuals) differed significantly from groups 7 (fish fed), 10 (fish fed with T. sirtalis), and 11 (worm fed with T. sirtalis). Group 11 also differed from group 8 (worm fed).

Between observations 2 and 3 there was a nonsignificant difference ($F(1,29)=3.13$, $p=0.094$) between litters with litter 39 increasing slightly (mean MSDIF=0.009 g) while litter 41 actually lost weight (mean MSDIF= -0.085 g). Diet also showed a nonsignificant difference ($F(1,29)=2.9$, $p=0.106$) in which worm fed snakes gained some weight (mean MSDIF=0.005) while fish fed animals lost weight (mean MSDIF= -0.071).

There was, again, a significant difference between MSDIF observed between measures ($F(2,89)=38.46$, $p<0.0001$) in which MSDIF from 1 to 3 averaged 0.24 g, with growth from observation 1 to 2 averaging 0.27 g, and from 2 to 3 averaging -0.04 g (a loss of mass). Mass increase over the first 85 days averaged 0.0029 g per day, while mass was lost over the next 146 days (average rate = -0.0002). The net weight gain over the whole 231 day period was only 0.001 g per day.

Percent Snout-Vent Increase (PSV)

PSV is a measure of relative growth based on the percentage increase of snout-vent length between two observations. From observation 1 to 3 there was a significant difference between the sexes ($F(1,29)=4.95$, $p=0.039$) with males increasing at a greater rate (mean PSV=0.175) than females (mean PSV=0.140). There was also a significant interaction between litter and sex ($F(1,29)=4.54$, $p=0.047$). Litter 41 males increased at a rate of 0.193, females at a rate of 0.135, while litter 39 males and females increased SVL at intermediate rates rate (0.153 and 0.144 respectively). The interaction between diet and sex was also close to significant ($F(1,29)=2.89$, $p=0.107$), with animals on the fish diet showing the greatest separation in growth (mean male PSV=0.18, mean female PSV=0.13).

Between observations 1 and 2 the increase in SVL was significantly different between litters ($F(1,37)=8.45$, $p=0.007$) with litter 41 growing at a rate of 0.146 over the first measurement, and litter 39 increasing by 0.109. Between observations 2 and 3 there was a close to significant effect of sex on PSV $F(1,29)=3.79$, $p=0.067$) with males increasing more (mean PSV=0.040) than females (mean PSV=0.024). There was a significant difference between observations ($F(2,89)=113.9$, $p<0.0001$). PSV from observations 1 to 2 averaged 0.13, from 2 to 3 averaged

0.033, and between 1 and 3 averaged 0.16, all three significantly different from one another.

Percent Mass Increase (PMI)

This is a measure of relative mass gain via the difference between observations. Between observations 1 and 3 only diet approached significance ($F(1,29)=3.13$, $p=0.093$), with worm eaters increasing more (mean PMI=0.169) than fish eaters (mean PMI=0.132).

Between observations 1 and 2 there was a significant difference in PMI between housing groups ($F(5,37)=2.84$, $p=0.035$). Group "12," individually housed animals, gained mass at the greatest rate (mean PMI=0.245), while both groups housed with T. sirtalis showed the least increase (mean group 10 PMI=0.079, mean group 11 PMI=0.055). This is shown in Figure 3.3. As with MSDIF, LSD Multiple Range tests discriminated group "12" (individuals) from groups 7 (fish fed), 10 (fish fed with T. sirtalis), and 11 (worm fed with T. sirtalis). Group 11 was also found to significantly differ from groups 8 (worm fed) and 9 (animals on each diet).

From observations 2 to 3 there was a significant effect of diet ($F(1,29)=4.44$, $p=0.049$) with worm fed animals increasing at an average of 0.029, fish fed animals decreasing at a rate of -0.034. There was also a significant difference between litters ($F(1,29)=5.86$, $p=0.026$), litter 39 increasing by 0.024 while litter 41

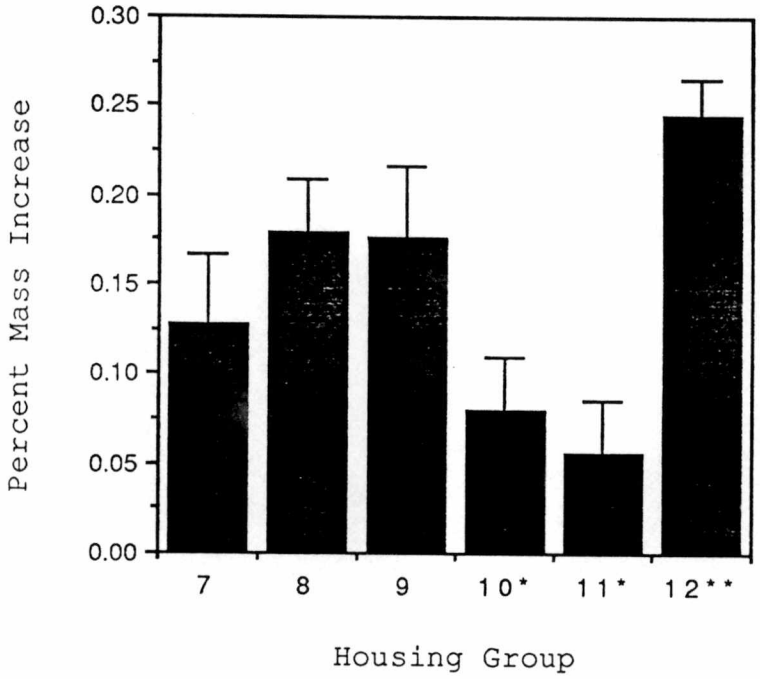

Figure 3.3. Percent mass increase (PMI) +1 standard error (SE) of T. butleri by housing group from observation 1 to 2.

Note: "*" denotes groups housed with T. sirtalis, "**" denotes individually housed.

lost mass (mean PMI= -0.033). A significant interaction between diet and sex also occurred ($F(1,29)=7.71$, $p=0.012$) where only male worm-eaters gained mass (mean PMI=0.055). There was a significant interaction between diet and litter ($F(1,29)=3.17$, $p=0.049$) such that only males from litter 39 increased mass (mean PMI=0.060) while females of litter 41 decreased the most (mean PMI= -0.075).

The difference between PMI between different observations was significant ($F(2,89)=35.51$, $p<0.0001$) with PMI from observations 1 to 2 averaging 0.154, from 2to 3 averaging -0.006, and from 1 to 3 averaging 0.148.

Condition Index (CI)

CI is basically a measure of volume such that higher values reflect a stouter body shape, lower values a more slender shape. At the initial observation there was a highly significant difference between litters ($F(1,37)=14.69$, $p=0.0007$), with litter 41 having a high CI of 0.096 and litter 39 a lower value of 0.084. At observation 2 there was a nearly significant interaction between diet and sex ($F(1,37)=3.14$, $p=0.088$) in which fish fed females and worm fed males had higher CI values (mean CI 0.075 and 0.074 respectively) than fish fed males and worm fed females which were identical (mean CI 0.069). There were no significant effects on CI at observation 3.

Time had a significant effect on CI ($F(2,89)=93.74$, $p<0.0001$) such that CI value decreased over time,

reflecting a "slimming" of body shape. Initial CI averaged 0.090, at 85 days it averaged 0.072, and at 231 days CI averaged 0.064, all of which differed from each other significantly. Figure 3.4 illustrates the CI over the 3 observations by litter. Table 3.2 (page 46) shows a summary of the important findings of these tests.

Discussion

Mass is generally a more variable measure than length; an animal can lose weight by decreasing body fat, but generally cannot lose length. Regarding the T. butleri in this study, it is clear that the main difference in mass is between the sexes, with males being heavier than females throughout the study. However, this difference only involves absolute mass in grams, not the measures of growth such as mass-difference (MSDIF) or percent mass increase (PMI).

The differences between measurements of mass show litter effects on growth, with litter 39 showing greater increases than litter 41 between the second and third observation. In fact, snakes in litter 41 actually lost some weight between the last two measurements. This tendency is also reflected by the percent mass increase, reflecting the percentage increase in body mass between the two measures. Diet also comes into play here, with worm-fed snakes showing a greater percentage mass increase

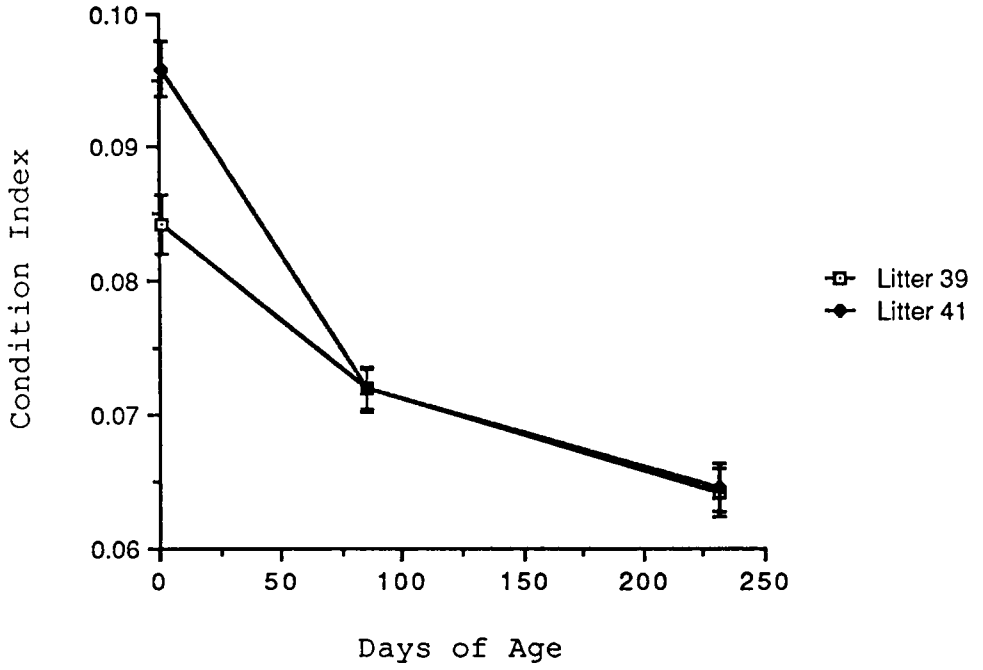

Figure 3.4. Condition index (CI) ±1 standard error (SE) of T. butleri over time by litter.

than those fed fish between the last two observations. The growth rates between the first and second observation showed a difference between housing groups in that the animals housed individually showed the greatest mass increase while those housed with another, larger, species, T. sirtalis, gained the least mass.

Snout-vent length (SVL) is a more conservative measure of growth since it can only increase at different rates but not decrease. The initial differences in snout-vent length of these animals lay between litters and sexes, with litter 39 being longer than litter 41, and males being longer than females. There was also a slight tendency for the animals that would later be fed worms to be longer than those to be fed fish. Note that the snakes of this worm-specializing species that readily accepted fish tended to be smaller than those that refused. However, only the difference between sexes persisted over the observations, though decreasing in significance.

Litter showed the greatest difference in growth rate (measured by either snout-vent difference or percent snout-vent increase) between the first and second measurements, with litter 41 demonstrating the greater increase. Litter 39 was the longer at first but litter 41 "caught up" in length by the end of the study. Also, when litter came into play regarding mass, it was litter 39

that increased more. This may be evidence that the two litters allocated growth in different ways.

Between the second and third observation the sexes showed a greater difference in snout-vent length increase, with males growing at a greater rate than females. Across all three observations, percent snout-vent increase was greatest for males, and in fact, for males of litter 41.

The strong effect of sex on growth in both length and weight is interesting, since it was the males showing greater growth. Scudder (1983) reports that female Nerodia grew more quickly than males. In adult snakes it is the females that attain larger sizes, with male growth decreasing earlier (Carpenter, 1952b). There is significant selective pressure on females to attain larger sizes and thereby increase number of offspring, while there are no such corresponding pressures on males (Semlitsch and Gibbons, 1982). In fact, males may benefit from remaining relatively small by having lower energetic costs of maintenance than the larger-bodied females. Crews et al. (1985) demonstrated that female T. sirtalis parietalis were larger than males within 3 weeks of birth, and that this difference was due to inhibitory effects of androgens, with castrated males growing at the same rate as females.

Andrews (1982) describes two patterns of growth in sexually dimorphic reptiles. One is for both sexes to

grow at the same rate with the smaller sex decreasing in growth rate earlier than the larger. The second is for the larger sex to grow at a consistently greater rate than the smaller sex. Only one counterexample is cited (from Blair, 1960) in which juvenile male Sceloporus olivaceus grew at consistently faster rates than females despite females attaining larger adult sizes. Scudder (1983) also mentions that changes in feeding regimen have greater effects on growth rate of females than males. It is possible that the constrained amount of food used in this study was sufficient for male growth but not for full growth capabilities of the females. There are few studies of growth in T. butleri that detail growth at very young ages, so it is difficult to fully explain this difference at this time.

The lack of impact of diet on growth in these animals is welcome for the purposes of the present research. Compared to the 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, in press). One would also expect a worm-specialist species to be more efficient at assimilating the diet it is presumed more adapted to. In fact, wherever differences occur between the diets, it

is in favor of snakes fed earthworms. However, T. butleri also fared well on the more unusual diet of fish, growing at a rate comparable to their counterparts fed earthworms.

The difference in growth rate between observations is also worth discussion. Clearly length increased over time, however, there is a great deal of difference between the rate of growth observed between the first two measurements and that occurring between the last two. It is possible that this is a natural effect of growth rate decreasing with the age and size of the animals, however, these animals were still quite young and small. Also, some animals actually lost weight between the last two observations, which would not be expected if a natural decrease in growth rate was the case.

It is possible that this is related to the decrease in available Gambusia toward the end of the study. In this case, it would seem that the effect of limited prey on this species is that as growth occurs it is put mainly into length. In such a case mass increase virtually stops, and may reverse. It would also seem that those animals fed worms were at an advantage, since those fed fish were the ones more likely to lose weight during this period even though meal sizes remained proportional. It may be that these worm specialists are better able to assimilate small meals of worm than fish.

Temperate zone reptiles have also been shown to have circannual rhythms. The turtle Pseudemys concinna has been shown to grow only between March and November despite favorable temperature and prey availability (Jackson, 1970). It is the case here that the period between the first and second observations included the months from July to October, while the last observation was taken the following April. Although light period, temperature and humidity are controlled for as much as possible, other individual snakes in the same lab consistently decrease prey acceptance during the winter months (Lyman and Layne, personal observation). It is possible that despite continued feeding there was an effect of a circannual rhythm upon the growth rate of the subjects over the winter months.

Another possible influence on the growth rate of these animals between the second and third observations may be stress. All testing procedures (aside from the two initial tests) were performed between the last two observations, which is the period of slowest observed growth. Supporting this idea is the observation that the individually housed animals gained the most weight during this period. This is in direct contrast to studies demonstrating that reptiles in groups gain weight at a greater rate than isolates (Burghardt, in press; Burghardt and Rand, 1985; Overmann, 1970; Tinbergen, 1953).

However, in the present study these were the animals being handled the least, since they were fed in their home container whereas grouped animals were moved twice weekly to small feeding containers to eat and then returned to the group. Also, the animals that showed the least growth were those housed with T. sirtalis, a larger species of garter snake which may occasionally eat other snakes (Lyman, personal observation), and which were also experiencing somewhat reduced meals. There were also fewer conspecifics in these two-species groups, and Burghardt and Rand (1985) showed that group size correlates positively with growth rate in young iguanas. If this is the case, it seems that males may be less susceptible to effects of stress on growth, which supports Scudder's (1983) report of the effects of change in feeding on female Nerodia, as mentioned above. It also would seem that animals feeding on the more natural diet of worms may have had some advantage over those on the more unusual diet of fish.

The condition index (CI) has been used in studies of hatchling quality in iguanas, in which higher values indicate hatchlings with greater yolk reserves or higher body fat (Werner, Packard and Paton, unpublished). In T. butleri the most noticeable trend in this variable is that it decreased over time, demonstrating a "slimming" of general body shape. This could be directly related to the

loss of stored yolk after birth combined with a greater proportional increase in length than weight as these animals grow. Since sexual maturity in most snakes is size, specifically length, dependent (Porter, 1972), this seems to be reasonable. There was also a difference in the initial measurement of CI between the litters, litter 41 being stockier, which may well be due to differences in retained yolk. Andrews (1982) states that snakes can grow without food for several days after birth relying solely on stored yolk. Indeed, it is this stockier litter 41 which showed the greatest growth in length over the course of the observations.

In conclusion, it appears that effects of diet on growth rate were minimal. Virtually all effects on growth were based on sex and litter. If it can be assumed that health and its effects on responsiveness are correlated with growth rate in neonate snakes, this study 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. butleri. This lack of effect of different diets on growth should indicate negligible differences in snake condition which may impact on other measures used in this research.

CHAPTER 4

CHEMICAL PREY PREFERENCES

Introduction

The vomeronasal system of a snake, comprised of the vomeronasal organ (VNO) in the roof of the snake's mouth and the forked tongue, is the single most important sensory system in finding and identifying prey items (Burghardt, 1966, 1967; Burghardt & Pruitt, 1975; Halpern, 1987; Halpern & Kubie, 1983; Wilde, 1938). It has been shown that tongue-flick rates correspond to a snake's activity level (Chiszar & Carter, 1975; Gove & Burghardt, 1983) and increase in the vicinity of natural prey items (Burghardt & Denny, 1983; Carr & Gregory, 1976; Chiszar, Scudder and Knight, 1976). The Tongue-Flick Attack Score (TFAS), which is a method of scoring interest based on tongue-flick rate and latency to attack, was developed by Burghardt (1966) and has been compared to other such measures by Cooper and Burghardt (1990).

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). Prey preference polymorphism has been documented within litters of garter snakes, in which different individuals will show preferences for different prey types (Burghardt, 1975).

Arnold (1977, 1981) has demonstrated that such differences in prey preference show a genetic basis. There is no effect of maternal diet on these responses (Burghardt, 1971).

Experience with various prey can affect changes in the initial preferences of garter snakes. Fuchs and Burghardt (1971) showed 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. Arnold (1978) subsequently demonstrated that experience with live fish increased later responsiveness to dead fish in neonate common garter snakes, T. sirtalis. Further, he established that animals fed exclusively on fish showed higher responses to chemical extracts of fish than did animals raised on frogs. The plains garter snake, T. radix, has been shown to exhibit such response shifts in chemical prey preference based on experience. Animals in the same litter raised on different diets of earthworm or fish shifted preference towards the experienced item regardless of initial response (Burghardt and Lyman, in preparation). This species is a generalist feeder known to eat both worms and fish in the wild.

Thamnophis butleri is, as described in Chapter 2, an earthworm specialist closely related to T. radix (Carpenter, 1952a; Conant, 1950; Ruthven, 1908). This

species is somewhat unusual in that naive snakes responded to worm, leech, fish and amphibian extracts (Burghardt, 1969) even though these animals are not known to feed on fish or amphibians in the wild. They have been reported to feed on fish in captivity (Halloy, 1987; Ruthven, 1908). In the following experiment, T. butleri were tested for chemical prey preference before and after experience with prey (either earthworms or fish) to investigate how such experience may affect the responses of a feeding specialist. Previous work would indicate that experience should shift initial preferences towards the familiar prey, but the responses of a dietary specialist may be more fixed than those of generalist species, such as those that were used in earlier research. The effects of litter, sex, and housing group were also investigated.

Methods

Subjects

Subjects were 38 T. butleri derived from 2 litters (39 and 41). Although 41 snakes were included in the initial test, only the 38 that survived for the duration of data collection are discussed. 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 below). Exact diet assignment of individuals was

constrained by acceptance of the offered prey as described in Chapter 2. Maintenance is also detailed in Chapter 2.

Extract Test Procedure

Testing consisted of presentation of cotton swabs dipped in surface extracts of prey or controls to each snake for 30 seconds, recording the number of tongue flicks (both total, and those directed at the swab) and latency to strike in case of predatory attack. The swab was held slightly to one side of the subject's snout at a distance of approximately 10 mm. This allowed for 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 during testing were discarded. Otherwise, swabs were freshened in extract between subjects and reused.

Three different stimuli were presented. Each stimulus was presented to all snakes once before the next presentation round was begun. These stimuli were: control (de-ionized water), nightcrawler (Lumbricus terrestris), and mosquito fish (Gambusia affinis). Surface extracts of prey items were prepared in a standardized manner (Burghardt, 1969). This entails placing 3.0 g of clean, dry prey into 10 ml of 60^{o}C distilled water and stirring gently for 2 minutes. The liquid produced is

centrifuged at 2500 rpm for 10 minutes, the clear supernatant then being frozen in sealed glass vials until use.

Animals were placed in testing containers the afternoon before testing was to begin to allow time to acclimate to their surroundings. All snakes were visually isolated from each other at all times. Before testing began, all water dishes were removed for the duration of testing to avoid distraction. Shelters remained in the containers unless temporary removal was necessary to access an individual.

Initial Test

Thamnophis butleri were initially tested for chemical prey preferance at 10 days of age. There were 41 total subjects tested, 20 from litter 39 and all 21 members of litter 41.

All animals were tested in their home containers. Testing began at 0900 hrs, and the time between presentation of stimuli was approximately 45 minutes. Stimuli were presented in the order: Control, Worm, Fish, Control. The temperature in the room was 24°C, the humidity 49%.

Final Test

Thamnophis butleri were retested for chemical prey preferences at 159 days of age. At this time there were

38 surviving subjects, 18 in litter 39 (10 fed fish, 8 fed worms) and 20 in litter 41 (9 fed fish, 11 fed worms).

Animals were tested in 15 x 30 x 9 cm clear plastic containers with an unabsorbant paper substrate. 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," to control for, and investigate, any order effects. Animals tested with different stimulus orders were balanced for diet, sex and litter. Animals had not eaten for one week prior to testing, the maximal between-feeding period. Test schedule and procedure was otherwise identical to that of the initial test. Temperature in the test room was 23°C, humidity was 48%.

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 in case of attack. This is reflected by the equation:

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

where "TFmax" is the highest number of tongue flicks directed to the swab. In this way, strikes count more heavily than tongue flicks alone 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 of the two days of stimulus presentation were analyzed, and the two orders of presentation were also 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.

F-W Score: A difference score, "Fish - Worm" (F-W), was computed by subtracting the TFAS for worm from the TFAS for fish for each animal. This value reflects the difference in response of an animal to the two prey stimuli regardless of the overall level of responsiveness.

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. Tukey Multiple Range tests were used to establish the location of significant differences within the ANOVAs. All analyses were performed using Statgraphics 3.0 (STSC, 1988).

Results

Initial Test

TFAS: There was a significant difference in response to the four stimuli ($F(3,151)=17.89$, $p<0.0001$), with lnTFAS toward the worm extract averaging significantly higher (mean lnTFAS=3.17) than that toward either fish extract (mean lnTFAS=2.48), or water control (mean lnTFAS=2.24 and 2.15 respectively). This is shown in Figure 4.1. Only 9 attacks were made, 8 of which were toward the worm stimulus.

F-W Score: Differences in response to the two prey stimuli were significantly different only between animals assigned to different housing groups ($F(5,37)=2.59$, $p=0.049$), with group 11 (worm diet) and group 10 (fish diet), both housed with T. sirtalis, having the most fish-biased and worm-biased responses, respectively. Figure 4.2 illustrates this. Overall, the responses reflected a general bias in favor of worm stimuli with an average F-W Score of -13.16.

Final Test

TFAS: Animals on the fish diet showed a significant difference in response to the offered stimuli presented in order 1 (CWFCFW) ($F(5,53)=10.26$, $p<0.0001$), as did those on the worm diet ($F(5,59)=6.94$, $p<0.0002$). In both cases, the familiar prey elicited higher responses than the

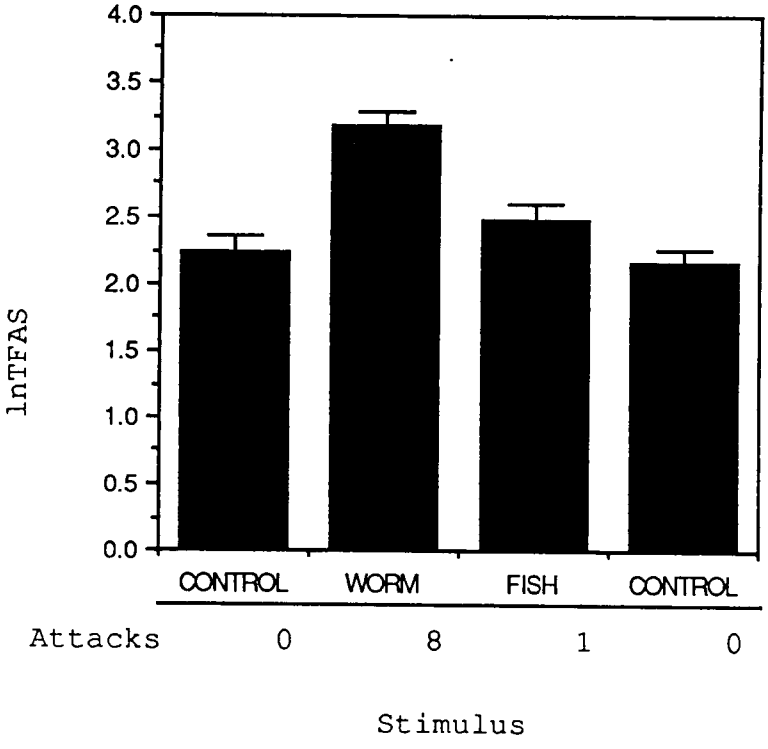

Figure 4.1. Log-transformed Tongue Flick Attack Score (lnTFAS) + 1 standard error (SE) results of Initial Chemical Prey Preference Test of Thamnophis butleri using surface extracts of worm (Lumbricus), fish (Gambusia) and water control.

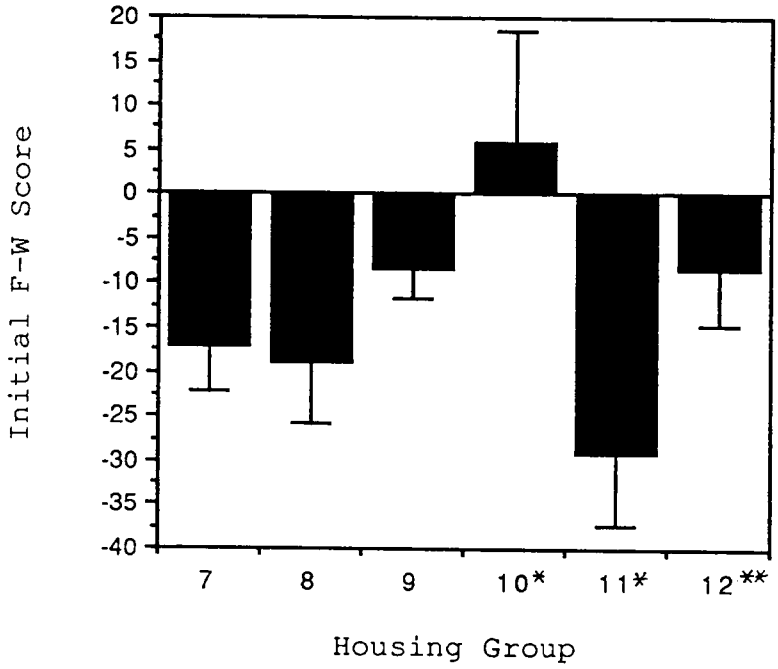

Figure 4.2. Fish - Worm responses (F-W Score) with standard error (SE) of different T. butleri housing groups in the Initial Chemical Prey Preference Test.

Note: "*" denotes groups housed with T. sirtalis,
"**" denotes individually housed.

unfamiliar, both receiving greater mean lnTFAS scores than water controls. Also, more attacks were consistently elicited by the familiar stimuli. Figure 4.3 details these results. Responses to order 2 stimuli (CFWCWF) were also significant for both animals fed fish ($F(5,59)=4.87$, $p=0.0019$) and those fed worms ($F(5,53)=17.52$, $p<0.0001$). Again, the familiar prey tended to have greater mean lnTFAS and a greater number of attacks than unfamiliar in both cases. A single individual in this test struck at all 6 stimuli presented, including both water controls, on one occasion without even emitting any tongue-flicks prior to striking. This animal was 410, a female fish-fed snake housed with T. sirtalis in group 10. These results can be seen in Figure 4.4.

There was also a significant effect of housing group on lnTFAS within both diets for each order of stimulus presentation. This is summarized in Figures 4.5 and 4.6. For the most part the tendencies still ran in favor of the familiar prey, but in the smaller sample size allowed in this analysis some extreme individual responses are apparent.

Utilizing only the first day's results, there were no significant effects of litter or sex on lnTFAS scores regardless of diet and/or order of presentation. There was a significant interaction between prey stimulus and diet ($F(2,113)=3.57$, $p=0.039$) in which animals responded

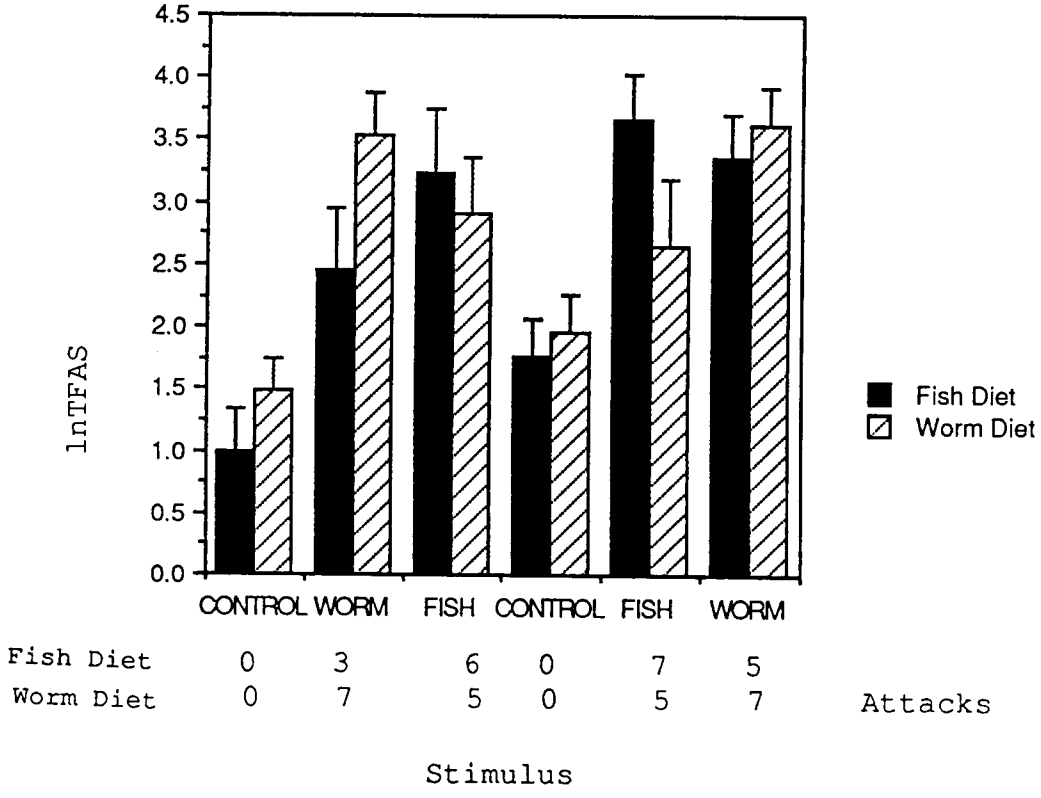

Figure 4.3. Log Tongue Flick Attack Score (lnTFAS) +1 standard error (SE) responses of T. butleri on different diets during Final Chemical Prey Preference Test to stimuli presented in order 1: control (water), worm, fish, control, fish, worm.

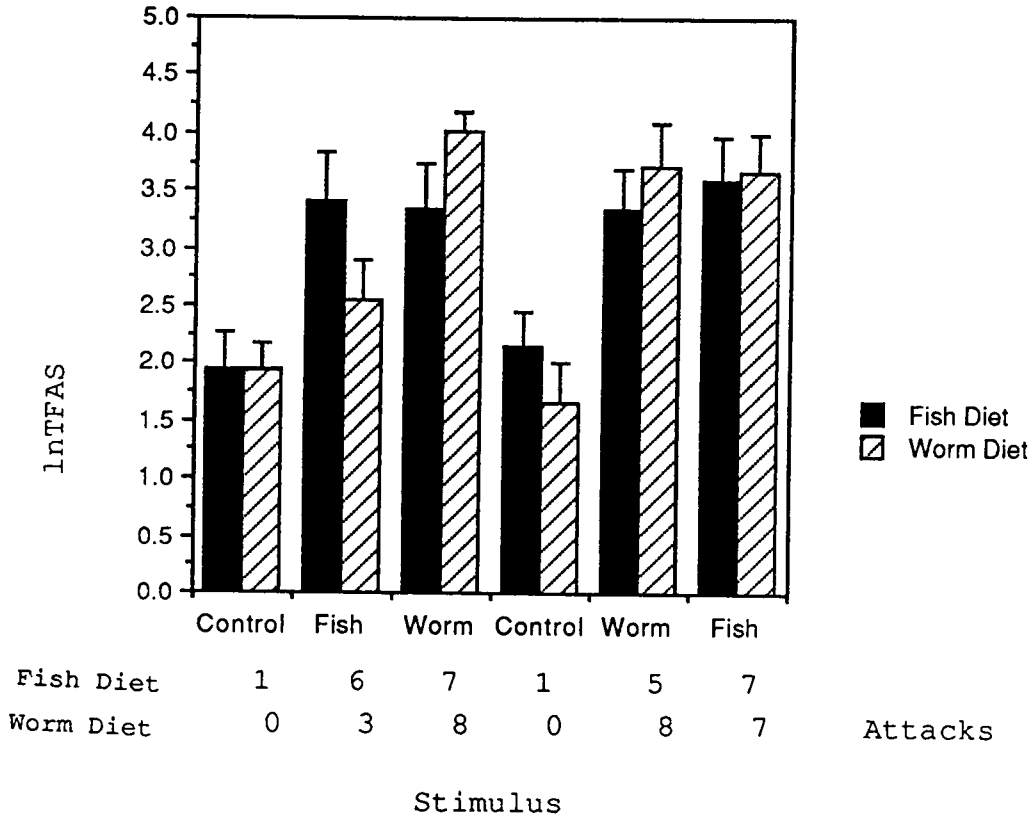

Figure 4.4. Log Tongue Flick Attack Score (lnTFAS) + 1 standard error (SE) responses of T. butleri on different diets during Final Chemical Prey Preference Test to stimuli presented in order 2: control (water), fish, worm, control, worm, fish.

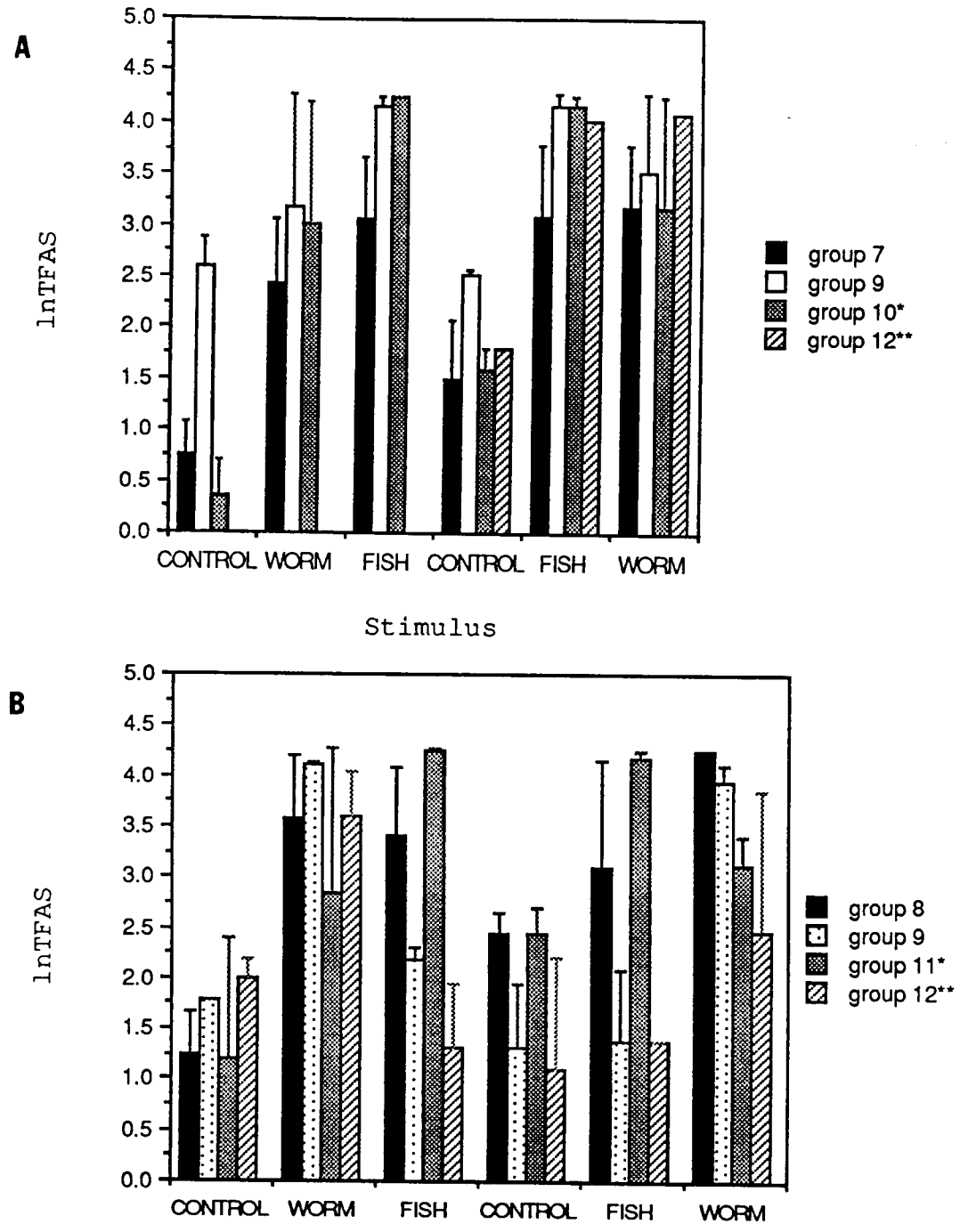

Figure 4.5. Log Tongue Flick Attack Score (lnTFAS) + 1 standard error (SE) responses of T. butleri in different housing groups for Final Chemical Prey Preference Test, stimulus order 1 (control, worm, fish, control, fish, worm), of animals on A) fish diet and B) worm diet.

Note: "*" denotes groups housed with T. sirtalis,
"**" denotes individually housed.

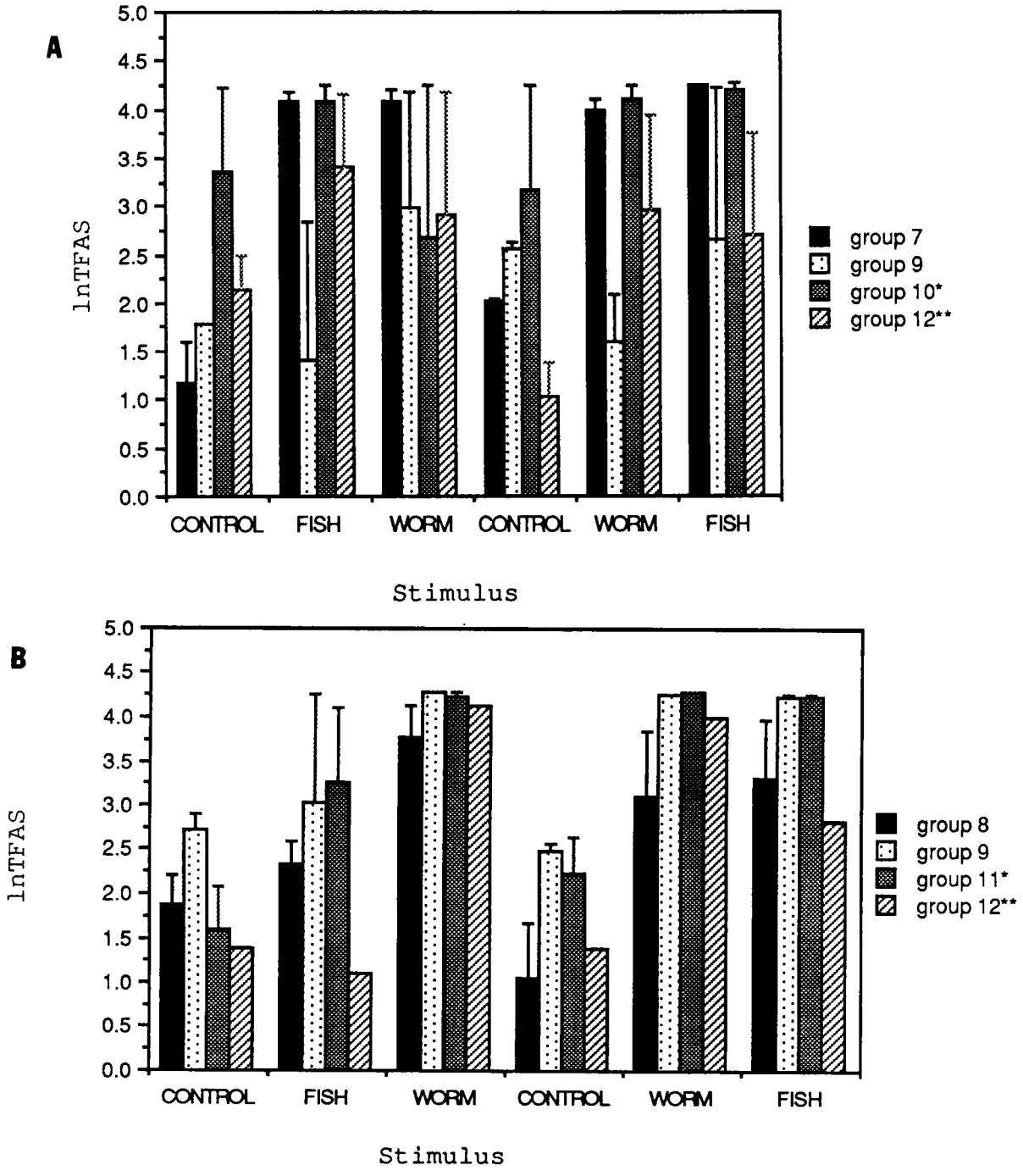

Figure 4.6. Log Tongue-Flick Attack Score (lnTFAS) + 1 standard error (SE) responses of T. butleri in different housing groups for Final Chemical Prey Preference Test, stimulus order 2 (control, fish, worm, control, worm, fish), of animals on A) fish diet and B) worm diet.

Note: "*" denotes groups housed with T. sirtalis,
"**" denotes individually housed.

with higher lnTFAS toward the familiar prey. See Figure 4.7

F-W Score: There was a significant difference in F-W score between animals on the two diets ($F(1,37)=8.48$, $p=0.007$). Fish fed animals preferred fish (mean F-W score= 9.87) and worm animals preferred worm (mean F-W score= -15.70). Figure 4.8 summarizes the effects of diet on both the Initial and Final tests. There were no other significant effects on F-W scores, but when analyzed separately by diet there were some nearly significant effects of housing group on worm fed animals ($F(3,18)=3.09$, $p=0.069$) and an interaction of group and litter in the fish fed snakes ($F(3,18)=3.74$, $p=0.079$). Figure 4.9 illustrates this effect of group.

Effect of stimulus order: The interaction between stimuli and order of presentation was significant for animals on the worm diet ($F(5,113)=2.73$, $p=0.029$), but not for those on the fish diet. This reflects the greater difference in response toward the fish and worm stimuli shown by the worm fed group as opposed to that shown by the fish fed animals. A comparison of responses to novel prey stimuli experienced after familiar prey cues shows higher mean lnTFAS than responses to the same novel prey cues presented prior to those of familiar prey. This can be seen in Figures 4.3 (page 76) and 4.4 (page 77).

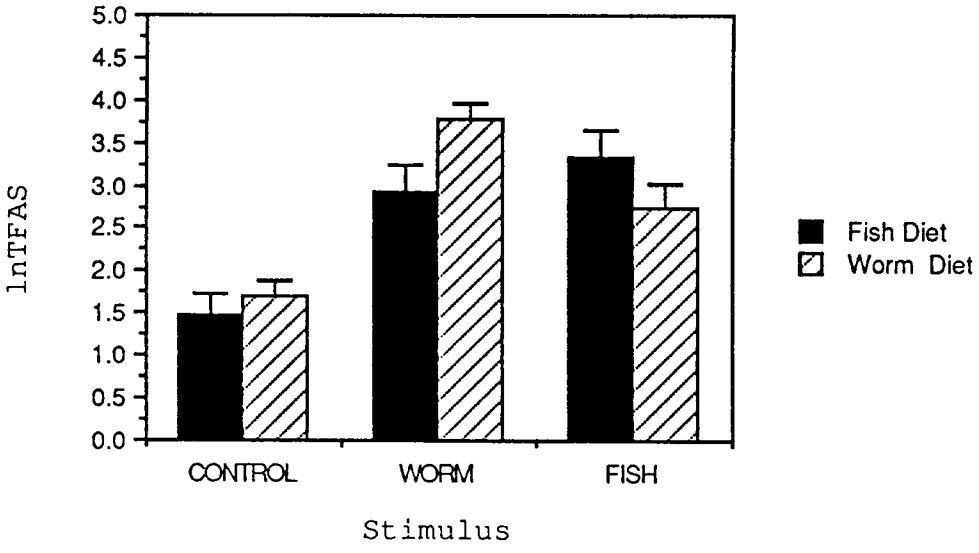

Figure 4.7. Log Tongue Flick Attack Score (lnTFAS) + 1 standard error (SE) responses of T. butleri on different diets during the first day of Final Chemical Prey Preference Testing toward the three stimuli: Water control, worm (Lumbricus), and fish (Gambusia).

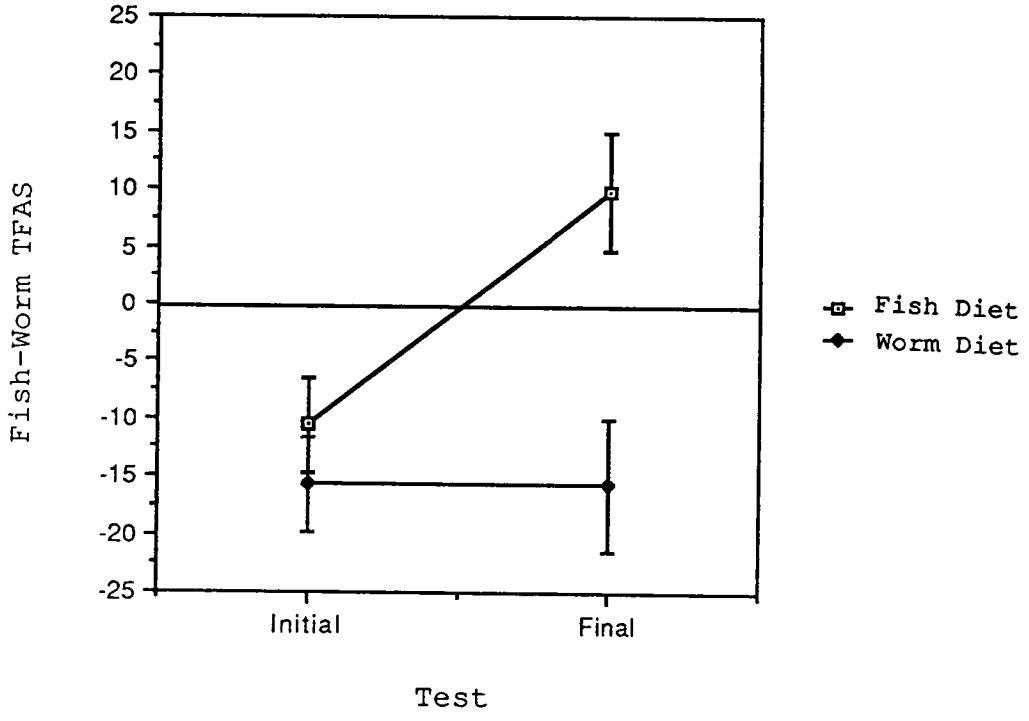

Figure 4.8. Fish - Worm responses (F-W) Score ±1 standard error (SE) of T. butleri on different diets between Initial and Final Chemical Prey Preference Tests.

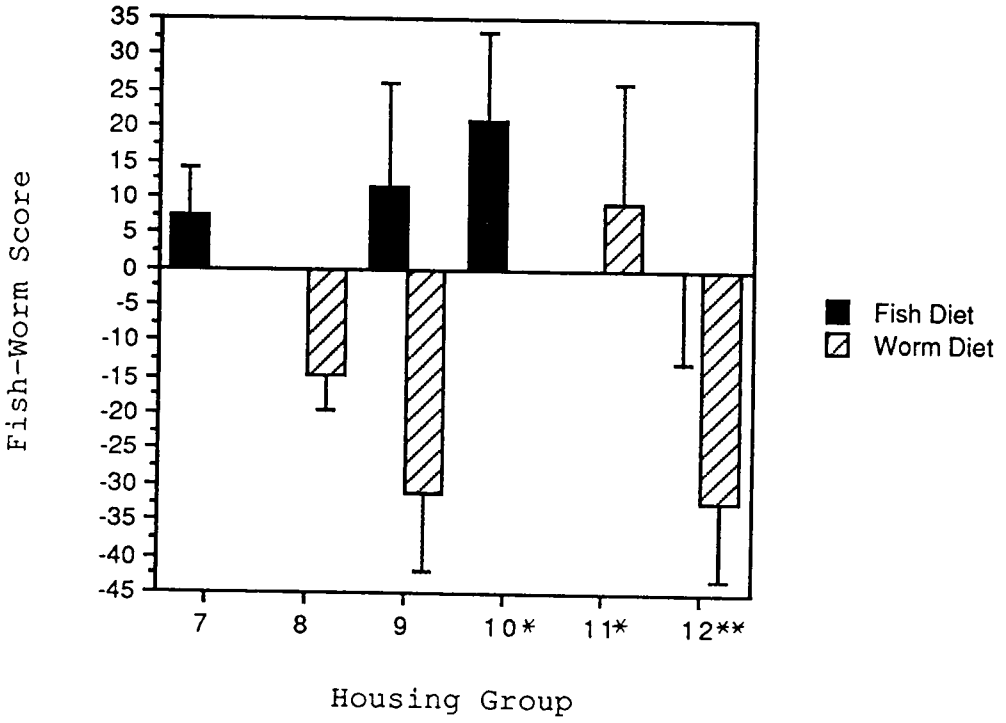

Figure 4.9. Fish - Worm responses (F-W Score) with 1 standard error (SE) of T. butleri on different diets by housing group in the Final Chemical Prey Preference Test.

Note: "*" denotes groups housed with T. sirtalis,
"**" denotes individually housed.

Comparisons Between Tests

TFAS: Repeated measures ANOVAs exhibited a significant difference in overall lnTFAS between the initial and final tests ($F(1,379)=5.64$, $p=0.019$) due to a general increase in responsivity toward the stimuli. This change was significant for the worm-fed animals ($F(1,189)=3.95$, $p=0.049$), but not for those fed fish.

F-W Score: There was a significant difference in F-W score between the two tests ($F(1,75)=4.49$, $p=0.049$), with the mean response moving from a strong worm bias (mean F-W score= -13.16) to an almost neutral average (mean F-W score= -2.91). This was due to the significant change in response of the animals on the fish diet ($F(1,37)=21.28$, $p<0.0003$) from a score of -10.59 (worm biased) to one of 9.87 (fish biased), whereas snakes fed worm showed no change at all. This is illustrated in Figure 4.8 (page 82).

Discussion

Prior to feeding experience, the T. butleri in this experiment showed a clear preference for earthworm extract over fish extract or a water control. This is expected based on data regarding natural prey. There were no differences in this response between sexes or litters. The proportion of animals later assigned to different diets were adequately balanced for initial preferences,

except for those assigned to groups 10 and 11 with T. sirtalis.

Experience with prey resulted in a dramatic change in response to fish extract in those animals fed fish. Snakes on the worm diet showed no change in preference between tests, but the response toward fish increased dramatically in those on that diet. The usual pattern was that animals on fish diets gave much higher responses to fish extract than the animals fed worms did, and somewhat lower responses to worm extract than the worm-eaters gave. Overall mean response was still slightly higher to earthworm extract than to fish. Even T. butleri that had never fed upon earthworms maintained a fairly high response to that prey, whereas those naive to fish never aquired much interest in piscine cues. This seems to be an ideal compromise between the previous work with more generalist species showing shifts in preference towards familiar prey (Arnold, 1978; Burghardt and Lyman, in preparation; Fuchs and Burghardt, 1971) and the dietary specialization seen in wild populations of this species (Carpenter, 1952a; Ruthven, 1908).

Comparison of the responses of the different housing groups is of some interest. In the initial test the individuals with more extreme responses appear to have been assigned to the two groups housed with T. sirtalis (10 and 11). This was probably due to a "left-over"

effect since animals housed only with conspecifics were assigned to groups first. For the most part preferences in the final test ran as expected, being higher toward the familiar prey. The exceptions to this, however, are rather interesting. The individually housed animals (group "12") demonstrate the lowest response levels of all the groups, mostly stemming from an extremely low response to fish extract by those animals fed worms. Also, those animals fed fish, though with a much higher response to fish than their naive counterparts, still showed a higher response to the unfamiliar worm extract. Could it be that there was some form of social facilitation involved in the increasing fish responses of the group-housed animals?

Another interesting point regarding the groups' responses to chemical cues involves those animals housed with a different species. In Chapter 3 it was reported that those T. butleri housed with the larger T. sirtalis did not grow as quickly as animals grouped only with conspecifics. It was suggested that stress due to being the smaller conspecific group and being housed with a larger, and possibly even predatory, species may have inhibited growth in these animals. Here we see that these same animals had the highest responses to chemical cues. One of these animals even struck at the water control. It seems possible then that these animals may have required larger amounts of food than their counterparts to achieve

the same relative growth, and that this may explain the higher responsiveness to prey cues seen in these animals.

In conclusion, T. butleri did show a marked initial preference for chemical cues derived from their natural prey, earthworms, which remained relatively stable over time regardless of experience. However, animals were capable of developing a preference through experience for the more novel prey of fish as well. There were no effects on these chemical preferences of litter and sex, and those effects of housing group seemed for the most part to also be related to dietary experience combined with individual variation.

CHAPTER 5

SITE CHOICE

Introduction

Snakes show preferences for chemical prey cues that correspond to experienced and natural prey items (Arnold, 1978; Burghardt, 1969, 1978; Lyman, the previous chapter). Many studies have demonstrated the ability of snakes to follow chemical trails of prey items utilizing the tongue-vomeronasal system complex (Chiszar and Radcliffe, 1976; Golan et al., 1982; Halpern and Kubie, 1983; Kubie, 1977; Kubie and Halpern, 1978; Noble and Clausen, 1936), and some have even used this behavior to evaluate learning abilities of snakes (Kubie and Halpern, 1975). Field observations have demonstrated that snakes are also attracted to habitats abundant in prey animals (Arnold and Wassersug, 1978; Kropach, 1971). In an experiment utilizing plains garter snakes, T. radix, animals were 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, in press).

Chemical cues regarding prey species are also available through less direct means than a prey animal's skin surface. Feces produced by garter snakes on differing diets can be distinguished even by human

olfaction, and may provide information regarding local prey to other snakes. This information may then be used in choosing foraging areas, and perhaps influence aggregative behavior.

Dundee and Miller (1968) were among the first to postulate that feces may be an attractant in snake aggregations. Several studies since have demonstrated that some snakes, including the plains garter snake T. radix as well as water snakes Nerodia sipedon and N. cyclopion prefer substrate soiled by conspecifics (Allen, Burghardt, and York, 1984; Porter and Czaplicki, 1974; Scudder, Stewart, and Smith, 1980). This preference would seem to lend credence to Dundee and Miller's (1968) suggestion.

Ward and Zahavi (1973) suggested the "information center" theory to explain how roosting birds seemingly shared information regarding the location of distant foraging sites. Galef (1986) subsequently demonstrated that rats were more likely to choose flavored foods that another rat had been observed feeding on, and that this information transfer was based on chemical cues. Porter, McFadyen-Ketchum and King (1989) have reported a similar finding in spiny mice, Acomys caharinus. The possibility that such "information" is used in choosing habitat sites will be explored here.

An earlier study with captive reared T. radix demonstrated that animals can distinguish between feces of conspecifics raised on different diets (Burghardt and Lyman, in preparation). Furthermore, the snakes showed site preferences based on these feces. These animals, as mentioned above, preferred sites marked with prey which agreed with chemical extract tests of prey preference. However, the same snakes showed a preference for sites marked with the fish-based feces. This was especially pronounced in the animals fed solely on earthworms. 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. However, 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 is a dietary worm specialist closely related to T. radix, as detailed in Chapter 2. In Chapter 4 the chemical prey preference of these subjects was reported, and found to be in favor of familiar prey, though the response to the natural prey of earthworm remained high regardless of experience. In the following experiment, T. butleri 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.

If this species can discriminate between feces based on the different diets, then they may utilize information transfer via feces. It seems likely that the strong natural interest in earthworms may attract this species to feces based on that diet rather than that based on fish. However, T. radix showed the opposite preference, and it may be that their close relatives T. butleri show the same pattern.

Methods

Subjects

Subjects were 38 T. butleri. Their history and maintenance is also detailed in Chapter 2. Although 41 snakes were included in the Initial Feces-Based Choice Test, only the 38 that survived for the duration of data collection are discussed. 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 below). Exact diet assignment of individuals was constrained by acceptance of the offered prey as described in Chapter 2.

General Site Choice Testing Procedure

All Site Choice testing was performed in visually isolated individual plastic containers with nonabsorbant paper substrate and "tent" shelters. 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. Depending on the test, 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, which was at about 1630 hrs. of the day before observations would be made starting at 0900 hrs. Figure 5.1 depicts this arrangement. Position 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.

Feces-Based Choice: Initial Test

Thamnophis butleri were tested for initial feces-based site choice at 11 days of age. Testing was

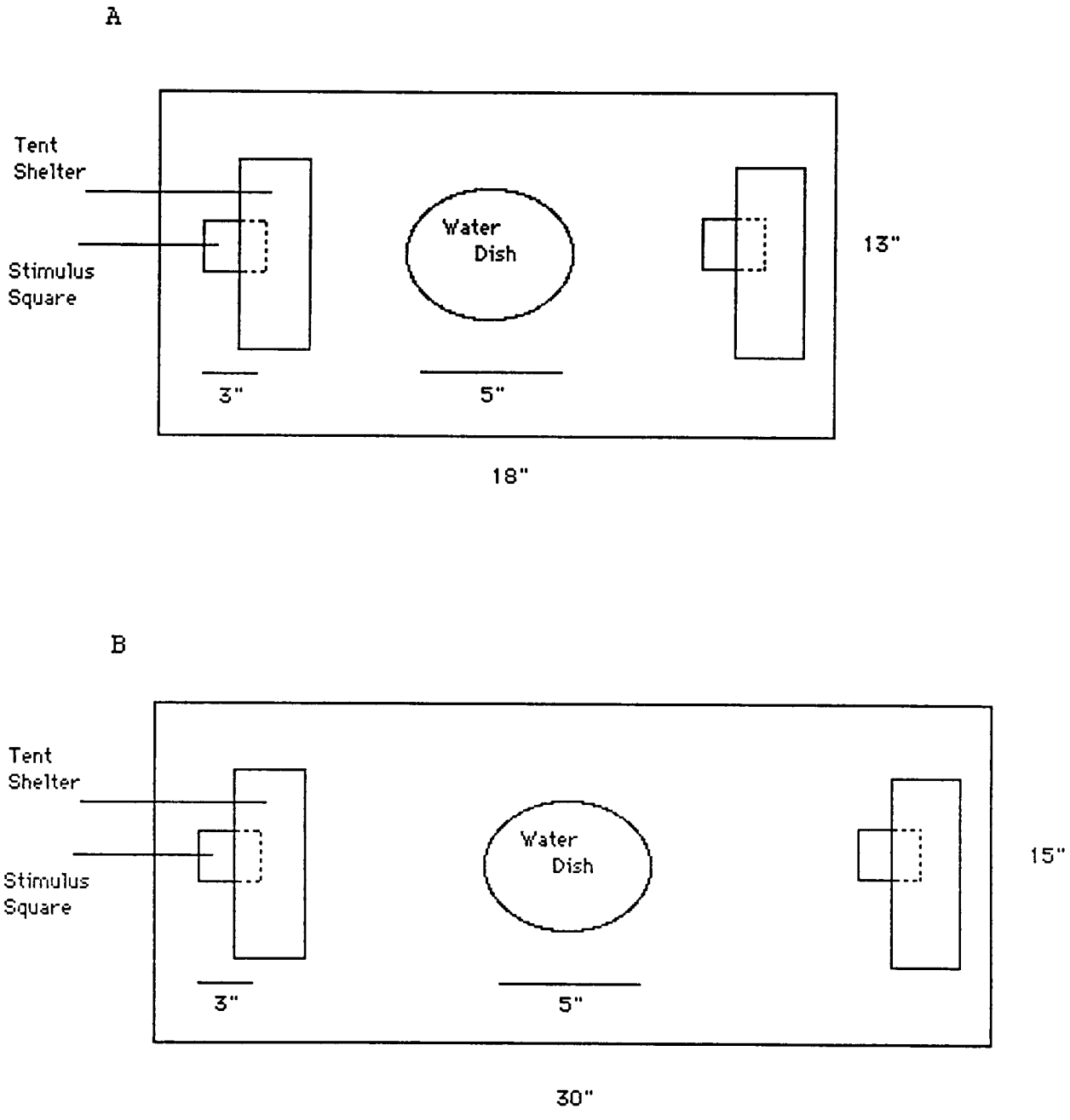

Figure 5.1: Thamnophis butleri Site Choice test boxes for A) the Initial Feces-Based Choice Test and B) the Prey Surface-Based Choice Test and the Final Feces-Based Choice Test. Distances are shown in cm.

performed in individual 13 x 18 x 5 cm containers as shown in Figure 5.1A. The stimulus squares were fixed to the substrate with a small amount of Elmer's © white glue.

In this test, stimulus squares bore feces from older, unfamiliar conspecifics from the same wild populations. Donors were fed only Gambusia or Lumbricus on each of at least four days after a 12 day fast before collection of feces was begun. Feces were collected several times daily and smeared with a wooden spatula directly onto the stimulus squares. 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 25.5±1°C, the humidity 52±3%. 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-Based Choice Test

Thamnophis butleri began Prey Surface-Based Choice tests at 122 days of age. Containers used in this test were of 15 x 30 x 9 cm clear plastic as illustrated in Figure 5.1B. In this test stimulus squares were cut from filter paper and fixed in place with double-sided cloth

tape. 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, totalling 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 butleri began the final test for feces-based site choice at 136 days of age. Procedures were identical to those for the Prey Surface-Based Choice Test

described above excepting for the stimuli. Stimulus squares were obtained in the same manner as in the Initial Feces-Based Habitat Choice test described above. Position of fish and worm cues were reversed in relation to the Prey Surface-Based Choice Test.

For the first 8 readings the daylight temperature was 23°C and humidity 46±2%. Due to a minor malfunction on the last day of testing, the chamber temperature rose to 25°C and humidity dropped to 24±2% for the last four readings.

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, 1956).

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 228 observations; Final Feces-Based Choice Test: 12 occurrences of 456 observations; Prey Surface-Based Choice Test: 6 occurrences of 456 observations). Instances in which different Choice scores summed to zero were also eliminated as ties (Initial Feces-Based choice test: 8 of 38 occurrences, Final Feces-Based and Prey Surface-Based Choice Tests: 5 of 38 occurrences).

Analyses were performed to compare the responses of animals of different litters, sexes, diets, and housing groups (and interactions thereof) among the experiments here performed. Responses to position of the container in the room (side rather than stimulus preference) were also subjected to Chi-square tests. All analyses were performed using Statgraphics 3.0 (STSC, 1988) except for the McNemar tests which were calculated as per Siegel (1956) using a hand calculator.

Results

Initial Feces-Based Choice Test

Responses were significantly biased in favor of worm-based feces (Chi-square=4.8, df=1, $p<0.05$), frequencies of choices being 55% of the individuals in favor of worm, 24% in favor of fish, and 21% with no difference. No significant effects of litter, sex, or assigned future diet were found on the responses to diet-based feces in

this test. Table 5.1 summarizes these results for this initial test.

After rotation of test boxes, 39% of the animals frequented the opposite stimulus side from the first three observations. In other words, they remained on the same side of the container relative to the testing room, although the stimulus squares had changed position. However, 53% of these animals were ones that had shown no preference for sides at all, and therefore had not been included in the statistical analyses of cue preferences. Of those 30 animals included in that data set, 23% of the animals showed preferences based on container side that were stronger than those shown for stimuli.

Prey Surface-Based Choice Test

There was no preferred prey surface cue, 42% of the snakes choosing fish cues, 45% worm cues, and 13% showing no difference. These results are seen in Table 5.2. However, individuals that preferred worm-marked habitats tended to show stronger preferences (more strongly negative responses) than animals that preferred fish marked habitats. There were also no significant effects of diet, sex, litter or group on choices based on Prey Surface.

Regarding tank position, 58% of the animals were significantly more often found in the back half of the box regardless of stimulus position ($t= -3.13$, $p<.0035$), only

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

Variable		N	Preference: Fish	Worm	None	Significance
Future Diet:	Fish	19	5	10	4	n.s
	Worm	19	4	11	4	
Litter:	39	18	5	8	5	n.s
	41	20	4	13	3	
Sex:	Male	20	4	13	3	n.s
	Female	18	5	8	5	
Total:		38	9	21*	8	*p<0.05

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

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

		Preference			
Variable	N	Fish	Worm	None	Significance
Diet: Fish	19	8	9	2	n.s
Worm	19	7	9	3	
Litter: 39	18	7	8	3	n.s
41	20	8	10	2	
Sex: Male	20	8	9	3	n.s
Female	18	7	9	2	
Total:	38	15	18	5	n.s

24% being found in the front and 18% with no difference. The greatest percentage of the snakes, 34%, were both associated with worm cues and the back of the container, though not significantly. Those subjects associated with fish cues showed no bias toward side of the test containers. There were no differences in the positions of animals on the two diets. However, only 15% of the 33 animals that showed any preferences based on stimuli showed stronger responses to tank side. Of side-based choices, 28% were made by animals that showed no preference to stimuli.

Final Feces-Based Choice Test

Diet showed a relationship to choices based on feces in this test. Frequencies of choices were not significant (Chi-square (1)=3.24, p=0.071), but 37% of the fish fed animals chose fish feces cues and 47% chose worm cues, while 63% of the worm fed animals chose fish feces cues and only 21% chose worm cues. Note these averages were in the opposite direction from diet, fish-fed animals were biased in favor of worm-based feces and vice versa. There were no significant effects of litter, sex, or housing group. Table 5.3 shows these results. Again, stronger preferences were obtained for worm stimuli (more negative values) than for fish.

With respect to which sides of the container were frequented regardless of stimulus position, 47% of the

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

		Preference			
Variable	N	Fish	Worm	None	Significance
Diet: Fish	19	7	9	3	*p=0.071
Worm	19	12*	4	3	
Litter: 39	18	7	6	5	n.s
41	20	12	7	1	
Sex: Male	20	10	9	1	n.s
Female	18	9	4	5	
Total:	38	19	13	6	n.s

Note: "*" denotes chi-square significance (3.24, df=1).

snakes were found more often in the rear of the tank, 32% in the front, and 21% showed no preference. Of animals found associated with worm feces, 77% were also in the rear of the tank, only 23% being more likely in the front, while those associated with fish feces were 40% more likely to be in the rear and 60% in the front (Chi-square (1)=3.88, p=0.049). There was no effect of diet on choice of container side. Thirty-Eight percent of the 32 animals that had shown preferences based on stimuli showed stronger responses based on tank position in this experiment.

Change Between Tests

Changes in preference between the Initial and Final feces choice test differed depending on diet of the animals. Fish-fed snakes showed no significant change in preference (McNemar Chi-square(1)= 3.60, p<.10). However, snakes fed worms showed a significant change from a bias towards worm-based feces towards a preference for fish-based feces (McNemar Chi-square(1)= 4.08, p<0.05). This change was evident in 59% of the 17 worm-fed animals that showed preferences in habitat choice based on feces cues. The remaining animals were evenly distributed in response. Figure 5.2 illustrates this.

Figures 5.2 and 5.3 may at first be difficult to interpret, but the utility of these graphic representations outweighs the inconvenience of a brief

explanation. Figure 5.2 presents information regarding the F-W Choice scores of animals on each diet in both the initial and final Feces-Based Choice Tests. This figure should be interpreted in the following manner: Positions of individual subjects' Choice scores are arranged in the vertical plane to correspond with responses during the Initial Feces-Based Choice Test, such that responses "higher" on the figure (more positive) reflect choices in favor of fish, while "lower" scores (more negative) reflect choices in favor of worm cues. Similarly, the horizontal plane corresponds with the responses to the Final Feces-Based Choice Test, with responses to the right (positive) in favor of fish and those to the left (negative) in favor of worm. To compare the responses between tests note the quadrant a response is positioned in, which are labelled for clarity; e.g. "worm first, fish second" meaning the subject's choice favored worm in the Initial test and fish in the Final. The more positive or negative a value is, the stronger the preference. Figure 5.3 is read in the same manner, but concerns the Prey Surface-Based and Final Feces-Based Habitat Choice Tests.

There were no significant relationships between habitat choices based on Prey Surface cues and those based on Feces-Based cues in the final tests. This is illustrated by Figure 5.3. The least likely combination of responses was a preference for fish prey cues and worm

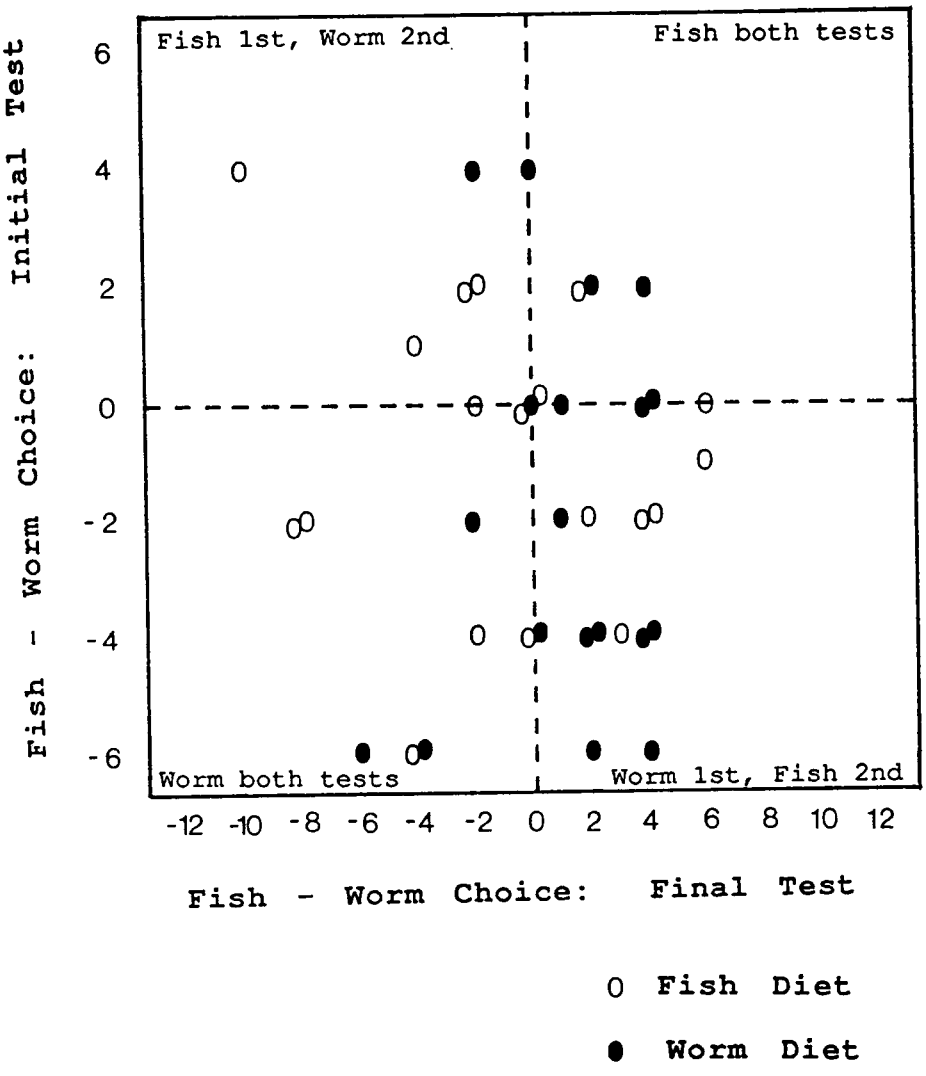

Figure 5.2. Relationship between Feces-Based Site Choices in Thamnophis butleri on different diets before and after dietary experience.

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.

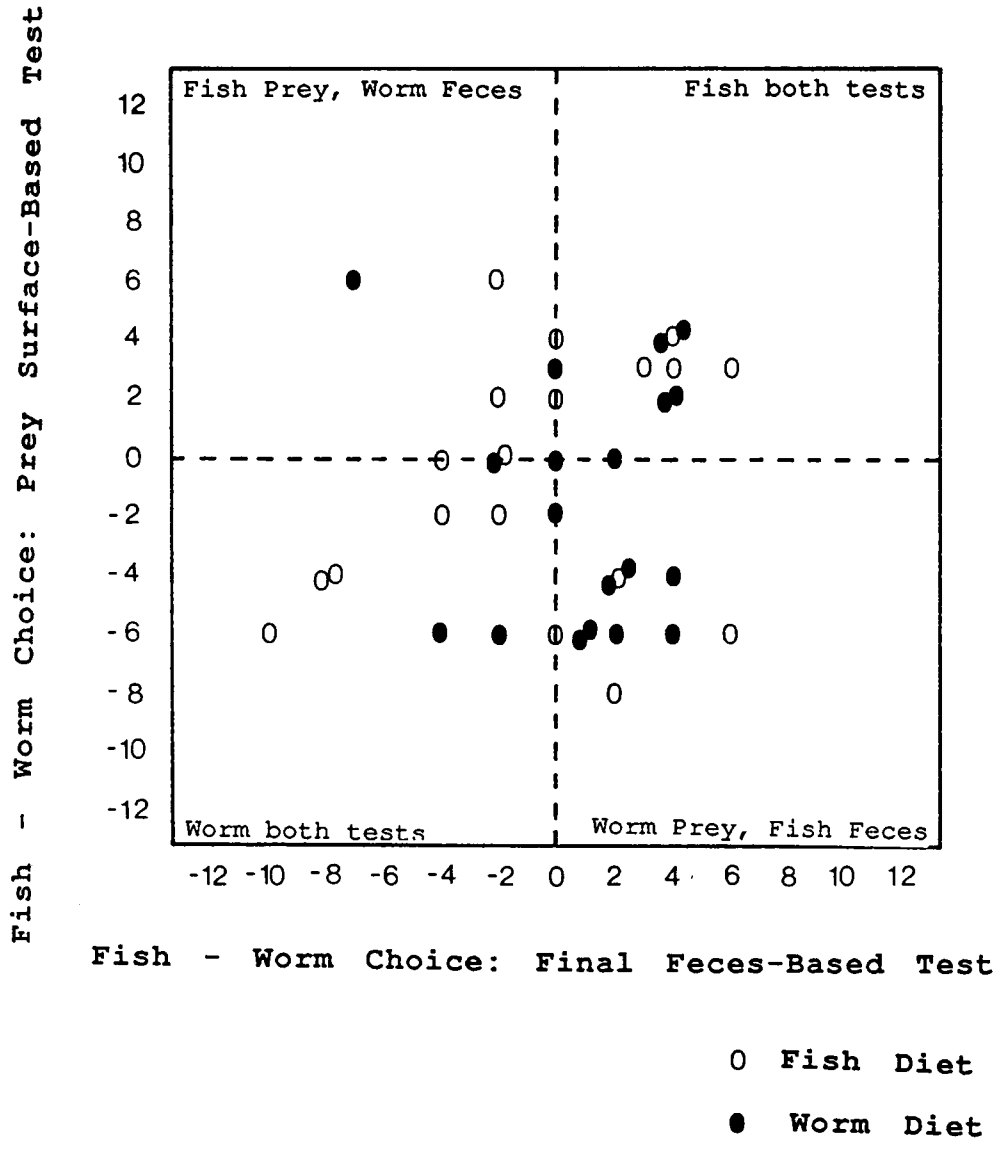

Figure 5.3. Effects of diet on the relationship among Prey Surface-Extract and Feces-Based Site Choices in Thamnophis butleri on different diets.

Note: Axes are as in Figure 5.2.

feces cues (3 occurrences). A combination of preferences for worm prey cues and fish feces cues was slightly more common (10 occurrences) than the fish/fish (8 occurrences) or worm/worm (7 occurrences) combinations.

Discussion

In these experiments the role of prey cues in site choice was assessed for Thamnophis butleri. These animals were tested using both direct prey cues (provided by aqueous surface extracts) and conspecific feces.

In the Initial Feces-Based Choice Test, carried out before the snakes had any feeding experience, the animals were shown to have a significant bias in favor of feces carrying worm cues. In the Final Feces-Based Choice Test, it was found that dietary experience had an effect on site choice such that snakes spent more time associated with the feces derived from the unfamiliar diet. For the animals that had been fed fish, this meant that the bias remained basically unchanged from the initial, that is, worm-based feces. However, the animals fed only worms changed their preferences to sites marked with the unfamiliar fish-based feces. The subjects showed no preferences for sites marked with prey cues based on diet, litter, sex or housing group.

Based on the ecology of T. butleri, one would predict that any site choice being made based on conspecific feces

cues would be in favor of those bearing information regarding the natural prey item, earthworms. It is quite surprising to see a bias towards feces carrying fish odors, especially in the animals that had never eaten fish rather than those that had. The close similarity of these results with the earlier tests with the related dietary generalist T. radix suggests that this is not an anomalous finding, and may reflect an ancestral phenomenon. Thamnophis butleri may retain the same response as T. radix even though it is no longer relevant since no conspecifics eat fish in the wild.

Similarly, one would expect snakes to prefer sites marked with extracts of the natural prey item. The generalist species T. radix has shown responses to cues of familiar prey in a similar experimental regime in which prey items were rubbed directly onto the stimulus squares (Burghardt and Lyman, in preparation). The lack of this response in T. butleri may be related to the predatory specialization of this species. Since this animal is not known to feed on fish in the wild, yet does in captivity, it may be that responsiveness to fish cues has not been lost, although it shows a very high sensitivity to dilute extracts of earthworm in addition to its natural preference for worm cues (Burghardt, 1967; Burghardt and Lyman, unpublished data). However, chemical cues regarding prey surfaces are apparently irrelevant in

habitat choice. The possibility that the extracts used may have unequal stimulus strengths seems highly unlikely based on previous research (Burghardt, 1969), and the fact that these same subjects had shown clear preferences toward the familiar prey in chemical prey preference tests using surface extracts, as was discussed above in Chapter 4. It is possible that there is a difference between extracts and prey surface "rubbings," such as was used with T. radix, that may account for this difference. These animals showed predatory interest in the same type of surface extracts, but perhaps some component required for site choice is not retained in the extract preparation process.

If it is true that cues based on prey extracts are not utilized in site choice by T. butleri, then it is possible that it is not the prey information per se that is of interest to these animals in choices based on conspecific feces. If these choices were motivated by some factor such as interest in an unfamiliar diet based on the greater nutritive value of a varied diet, it seems likely that it would be reflected in choices based on the extracts of the prey as well. This, however, has been shown not to be the case.

It is also possible, of course, that somehow dietary information carried in feces differs from surface extract in some way that the snakes can detect. That is, perhaps

dietary cues in feces are broken down into different component parts than those in surface extracts, allowing animals to make choices based on things such as actual calcium content or richness in vitamin B of the diet fed upon, rather than what the prey species was. This possibility is intriguing, but far beyond the scope of the present study to investigate.

Gillingham, Rowe, and Weins (in press) have recently demonstrated in field observations that T. sirtalis use earthworm castings (feces) in locating prey. This has not yet been tested for in T. butleri, though it would seem reasonable for a worm specialist to be able to use these cues at least as well as a generalist feeder. Any similarities between feces produced by earthworms and that produced by snakes eating earthworms are unknown as well, but may provide an interesting comparison in the study of this behavior.

It is interesting to note that when investigating association of snakes with the sides of test boxes relative to external cues, rather than stimuli, it is the animals associated with worm cues (both feces, and to a lesser degree extract) that show a greater tendency to remain on the same side of the tank when rotated regardless of stimulus position. These have already been shown to be most likely fish-fed animals whose bias did not change from the initial test. Animals associating

with fish-based cues showed no side-bias. In the tests conducted in the environment chamber, animals may have preferred the rear of the container due to lower light levels, or possibly due to being farther from the experimenter during observations. Therefore, animals that developed an interest in sites marked with fish-based cues were more likely to be basing their choices on those stimuli, whereas animals choosing sites marked with worm-based cues may have been more influenced by external factors.

Another possible factor in site choice is one based on familiarity. Chiszar et al (1980) demonstrated that snakes exhibit more investigatory behavior and defecate sooner in a cleaned cage than an uncleaned one, which was interpreted as evidence that snakes respond to the absence of familiar odors. It was reported that snakes did not discriminate between their own and conspecific fecal materials, but also noted that these subjects were individually housed and did not have access to feces of other individuals. This may reflect habituation to a familiar cue, or an increased interest in novel ones.

A similar situation is described in the red-backed salamander, Plethodon cinereus, where animals utilize feces marked with pheromones in territorial behavior. These salamanders showed more defensive behavior and spent less time in burrows associated with same-sex, conspecific

feces than burrows marked with their own or "imitation" feces (Horne and Jaeger, 1988; Jaeger, 1986; Jaeger et al., 1986). This species of salamander has been shown to respond to familiar conspecifics with lower rates of aggression than that shown to strangers (Jaeger, 1981; Jaeger, 1986).

Most snakes in the present study were housed with other snakes fed the same prey. The familiar feces cues (their own and others) would be therefore based on the same diet. It is then possible that the prey information contained in feces was used as a "tag" to identify familiar animals rather than prey. Spiny mice have been shown to associate more with animals fed the same diet even if the particular animal was unfamiliar (Porter, McFadyen-Ketchum and King, 1989), which may be an example of this same idea.

Since the preferences shown were for feces based on the opposite diet, these snakes may be attracted to unfamiliar conspecifics. Such an attraction, with differences signalled by feces containing unfamiliar cues, could serve to have snakes associating with animals from different litters. That opens the possibility of selective pressure in which snakes are seeking to aggregate, and perhaps later mate, with unfamiliar and as such perhaps genetically variable animals.

This is further demonstrated by looking at the isolated animals (group "12") and those housed with conspecifics on the opposite diet (group 9). Five of the six isolated animals preferred sites marked with feces from the opposite diet. However, three of the four fish-fed animals in group 9 preferred the fish-based feces: the only condition in which animals showed a preference for feces based on the familiar diet.

This information may actually help to clarify why animals on the worm diet showed such a strong tendency to prefer fish-based feces. In the wild, T. butleri feed upon worms, so conspecific feces should be "expected" to carry worm-based cues. However, fish is a novel prey item, possibly making it a much more noticeable component in unfamiliar feces. Worm-fed snakes have never experienced feces carrying fish cues, therefore, such feces MUST belong to an unfamiliar animal. However, fish-fed snakes may have some natural "expectancy" of worm cues in conspecific feces, even if never experienced, making this a less obvious clue to familiarity.

Animals housed alone had only their own feces to compare others' to, so their choices are similar to those of animals housed with others on the same diet. However, the animals housed with animals fed the opposite diet showed some ambiguity in response. Animals fed fish here had been exposed to feces based on both fish and worm, and

chose sites marked with feces based on the diet eaten. Conspecifics in this group were also producing the "expected" feces derived from worm. Could it be that, given experience with both, fish-based feces remains the more novel due to the lack of its occurrence in nature?

The only problem with this hypothesis is that it doesn't so neatly explain the same tendency in T. radix for worm-fed animals to show the strongest preference for fish-based feces. These animals supposedly feed on both prey in the wild. However, all of these described experiments have been performed with neonate animals, and there is evidence that young generalist Thamnophis do feed more heavily upon earthworms than older conspecifics (Carpenter, 1952a). It is also possible that the population of T. radix used may have been more prone to feed on earthworms in the wild than would be imagined based only on their species-typical responses to chemical prey cues. Supporting this idea, Kephart (1982) has demonstrated that site was 5 times better a predictor of diet than was species of snake, based on populations of the generalist species T. sirtalis and T. elegans in California.

In conclusion, T. butleri have now been shown to initially show preferences for sites marked with feces produced by conspecifics feeding on the natural diet of worms. This pattern can change after experience with prey

and/or feces to a preference for novel feces cues. This tendency is demonstrated best by animals fed earthworms who more often prefer sites associated with fish-based feces. The lack of site preferences based on prey cues implies that the observed feces-based preferences are due not to the prey cues themselves but perhaps for the information regarding familiarity that dietary cues in feces may provide.

CHAPTER 6

AGGREGATION

Introduction

Although not generally thought of as "social" animals, many snakes show a tendency to aggregate (Barbour, 1962; Dundee & Miller, 1968; Kropach, 1971; Noble & Clausen, 1936). Heller and Halpern (1982b) have implicated the vomeronasal system in grouping behavior in adult male Thamnophis, and Burghardt (1980) showed comparable results with neonates.

Various functions and benefits of aggregative behavior in snakes have been postulated. Reduced oxygen consumption and water loss, and thermal stability have been demonstrated in Storeria dekayi (Noble and Clausen, 1936) and Boa constrictor (Myers and Eells, 1968). Protection from predation (Gregory, 1975), enhanced foraging (Barbour, 1962; Kropach, 1971), and increased growth rate (Burghardt, in press) have also been suggested. It has also been postulated that snakes in aggregations could be engaging in information transfer (Burghardt, in press; Burghardt and Lyman, in preparation).

Several influences on aggregation have been suggested in other species. As noted previously, Porter, McFadyen-Ketchum and King (1989) have shown diet to influence

association in spiny mice, Acomys cahirinus, through olfactory cues. In their study, mice preferentially associated with other individuals that had been feeding on the same diet. This occurred unless they were rendered anosmic, in which case they showed no discrimination.

There are of course other factors beyond diet shown to influence grouping behaviors. For example, social preference for siblings has been demonstrated in Japanese quail chicks, Coturnix c. japonica (Waldman & Bateson, 1989), even over nonsiblings raised together. Porter, Matochik and Makin (1986) demonstrated kin preference in spiny mice that was accentuated by familiarity such that familiar siblings were preferred over unfamiliar littermates, both of which were associated with more frequently than any nonsiblings. White-footed mice, Peromyscus leucopus, were then shown to prefer familiar animals regardless of genetic relationship (Halpin & Hoffman, 1987). Age and sex have been shown to influence grouping behavior in 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).

To investigate the influence of diet, kinship, sex, and familiarity on aggregative behavior, the grouping patterns of 24 T. butleri were observed in a large arena with several identical shelters. The subjects were

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 unfamiliar prey.

Thamnophis butleri has been described as a snake that forms aggregations outside of hibernation and reproductive periods (Carpenter, 1952a; Noble and Clausen, 1936), so aggregations were expected to form nonrandomly under otherwise identical shelters in this experiment. Factors of import in aggregation would be identifiable by the association of animals within the experimental design, and may include litter, sex, diet or familiarity. Possible preferences among litters, sex or degree of familiarity are unknown in this species, and vary in other taxa. Based on their choices of sites marked with feces of conspecifics on different diets, as described in Chapter 5, it is hypothesied that T. butleri will prefer to aggregate with animals on unfamiliar diets.

Methods

Subjects

Thamnophis butleri housed only with members of their own species (groups 7,8,9, and 12 [isolates]) began testing at 219 days of age. Eleven animals from litter 39

(Worm diet:3, fish diet:8) and 13 from litter 41 (worm diet:7, fish diet:6) were tested. Table 6.1 summarizes the diet, sex, litter, and housing group of each subject. Maintenence of these animals is described in Chapter 2.

The numbers of test animals reported here are decreased from the earlier testing sessions due to subject mortality. It is believed that toxic Lumbricus caused the deaths of these animals, as was detailed in Chapter 2. All 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 cycle (lights on at 0800 hrs.) and a 9:15 warm:cool cycle (23^{o}C starting at 0900 hrs, 18^{o}C starting at 1800 hrs.). Temperature changes were gradual over the course of about 45 minutes.

An oblong, galvanized metal water trough was used as the testing arena. This is depicted in Figure 6.1. 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.

Table 6.1: Composition of Thamnophis butleri groups used in arena aggregation test.

GROUP	DIET	LITTER	SUBJECT MALE	SUBJECT FEMALE
7	FISH	39	A, N	F, K
		41	P	I, L
8	WORM	39	P	E, R
		41	N, R	U
9	FISH	39	T	S
		41	C	M
	WORM	39	-	-
		41	S	-
"12"	FISH	39	B	O
		41	-	D
	WORM	39	-	-
		41	G, H, T	-

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

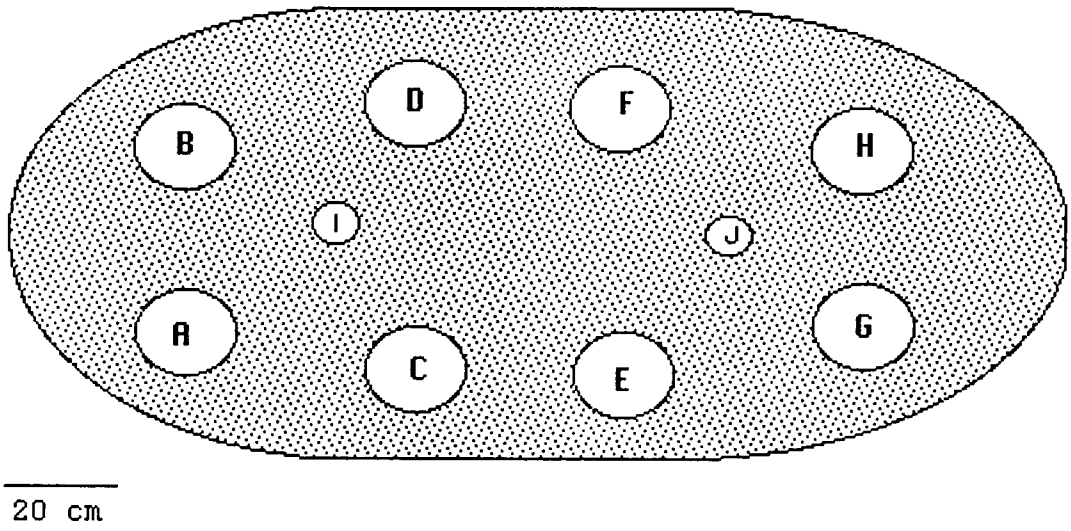

Figure 6.1. Diagram of arena used in observations of aggregation in T. butleri. Circles labeled A - H represent cover objects (16 cm diameter), circles I and J represent water dishes (10 cm diameter).

Cover objects, shown in Figure 6.2, 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 object. Cover objects were 16 cm in diameter and labelled A through H, corresponding to position within the arena as can be seen in Figure 6.1. These covers had been successfully used in previous aggregation experiments (e.g., Schwartz, 1989), and were modified only by the attachment of the wooden portion of the "feet" to sufficiently raise them above the gravel substrate. 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 tub.

Test Procedure

After being fed early in the morning, the snakes were introduced to the arena at about 1630 hrs. on the day

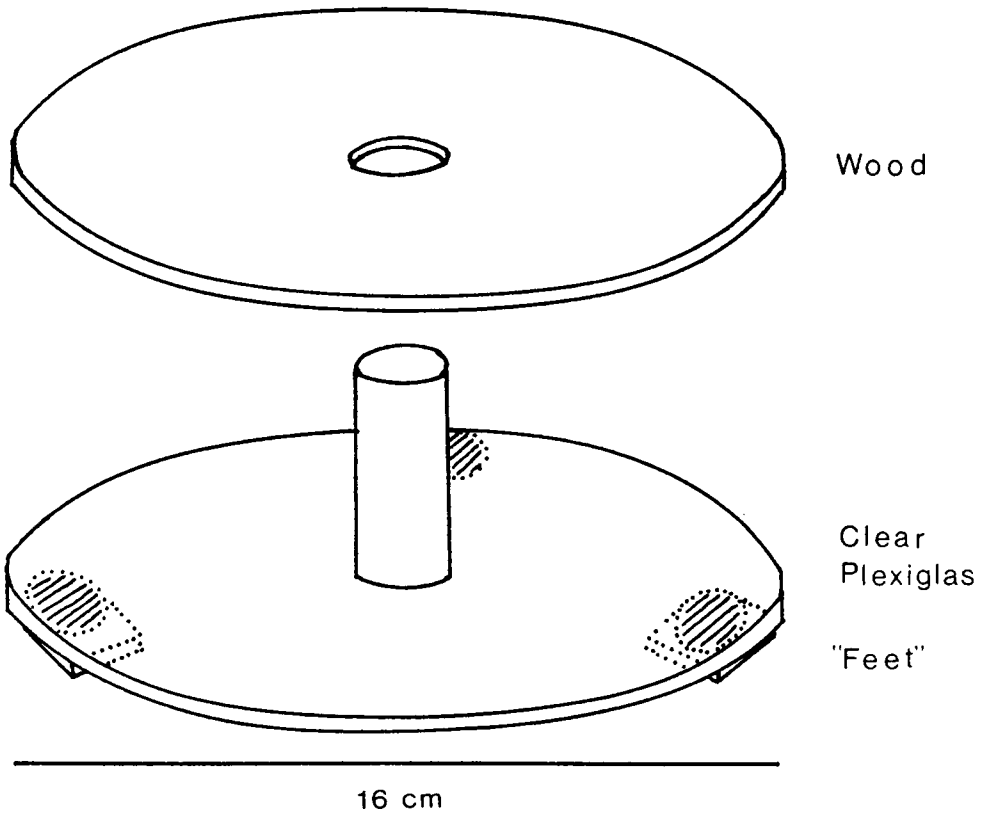

Figure 6.2. Cover objects used in arena aggregation tests of T. butleri.

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. Observations made of T. sirtalis in an earlier aggregation experiment 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, personal observation). A total of 192 snake observations (24 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) Occurrence of aggregation; B) Nearest Neighbor Distance (NND); C) Overall Nearest Neighbor (ONN); and D) Incidence of Co-occurrence. These are defined as follows.

Occurrence of aggregation. 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 and what shelters were used most often.

Site fidelity of the subjects was also tested, as were occurrences of physical contact between snakes.

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, which reflect the individual averaging the least distance from each subject over all 8 readings.

One thing to note is that very close proximity was more prone to error simply due to space taken to record each animal's ID code onto data sheets: 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. 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 which were produced for Pair NNDs (above) were used to determine for each subject the closest individual 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 co-occurrence (CO). This was measured in three different ways utilizing the number of readings that each possible combination of two animals were: 1) in direct contact with each other; 2) in the same continuous mass of touching snakes; and 3) found under the same shelter.

Statistical tests. Binomial probabilities were generated for all but the comparison of Pair NNDs, which were analyzed by average linkage cluster analysis (Sokal and Michener, 1958). Binomial probabilities were 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. All analyses were performed using SAS (SAS, 1985).

Results

Occurrence of Aggregation

Covered versus uncovered. While shelters (covered areas) constituted less than half the area of the arena, snakes were found to be under the shelters in 91%, and out in the open only 9% of the observations. This is a highly significant difference (8 shelters by 8 readings as baserate 1/64 (0.0156), total observations as trials 192, $p<0.00001$). Of the animals found in the open, 50% of the observations occurred during reading 5. Table 6.2 includes this information.

Aggregation. As compared to a binomial distribution, on each of 8 observations subjects were significantly clumped under shelters (shelters as baserate 1/8 (0.125), $p<0.03$), demonstrating aggregative behavior. This is detailed in Table 6.2.

There was a significant difference between the litters regarding cover use (Chi-square =4.20, df=1, $p=0.040$) in that only 27% of litter 39 snakes were ever found in the open, while 69% of litter 41 animals were uncovered at least once. Only three animals were found uncovered more than once, and these were all animals in litter 41. These three snakes, two of which were male,

Table 6.2: Number of T. butleri under each cover or in the open for the 8 aggregation readings.

	Shelter								
Reading	out	A	B	C	D	E	F	G	H	*sig
1	4	0	2	1	2	4	0	10*	1	p<.0001
2	1	5	2	0	0	1	0	11*	4	p<.000001
3	0	0	1	0	1	0	2	15*	5	p<.000001
4	1	0	5	1	0	3	1	9*	4	p<.001
5	9	0	1	2	0	0	5	6*	1	p<.03
6	2	0	4	3	1	2	1	8*	3	p<.001
7	1	1	0	1	1	2	6*	12*	0	p<.000001
8	0	2	5	1	0	3	1	10*	2	p<.0001
Total:	18	8	20	9	5	15	16	81	20	

Note: "*" illustrate significant differences, "*sig." reports the level of significance. Refer to Figure 6.1 for positioning of shelters A - H in the arena.

were found in the open a maximum of three times and were all on worm diets. It should be noted that of the 4 snakes of litter 41 that were never found uncovered, 3 were also worm-eating males, so an effect of diet on being under covers or in the open is not suggested. Snakes in the open were never in physical contact with each other.

Preferred shelters. Any snake utilizing the same shelter on 3 or more readings was showing a significant site fidelity (shelters as baserate 1/8 (0.125), snakes as trials 24, $p<0.02$). In this experiment, 79% of the animals were found under the same shelter at least 3, and as many as 7 times (values of 3 or more, $p<0.0001$). Animals were found under an average of four different shelters over the course of the observations, though subjects were only found under one shelter more often than twice.

Animals were found under one particular shelter in 47% of the observations. All groups under this shelter were significantly clumped. This shelter, "G," averaged 10.1 snakes per reading period (range 6 to 15), whereas the other shelters averaged 0.75 to 2.5 snakes each per reading. This can be seen in Table 6.2.

Nearest Neighbor Distances (NND)

Mean NNDs for each snake over the 8 readings ranged from 2.44 cm to 13.0 cm, averaging 5.81 cm. This

information is included in Table 6.3. These means were slightly less for litter 39 (mean NND=4.74 cm) than litter 41 (mean NND=6.72 cm). Animals on the fish diet (mean NND=5.22 cm) and males (mean NND=5.50 cm) also averaged slightly smaller separation distances than worm fed animals (mean NND=6.64 cm) and females (mean NND=6.18 cm). Student's t tests found no significant differences between these means.

Another way to consider this involves the mean distances between each possible pair of snakes over the 8 readings (Pair NND). This ranged from a minimum of 15.8 cm to a maximum of 89.6 cm. The mean for all combinations was 47.3 cm. The maximum mean distance between any snake and its nearest neighbor across all observations was 33.7 cm, with the average NND being 23.6 cm. Mean NNDs between animals of each diet, litter, sex, and group showed no significant differences, and were so similar to the overall mean that they will not be listed here.

Eight specific pairs emerged as consistent groups in an average-linkage cluster analysis, as shown in Table 6.4. All 8 of these primary pairs consisted of unfamiliar animals, 5 of which were also on different diets. Six of the eight pairs consisted of animals from the same litter, but sex showed no effects.

Table 6.3: Description of subjects and summary of measures of association: NND (nearest neighbor distance), Pair NND (least distance between individuals over 8 readings), and Co-occurrence with specific other individuals under the same shelters. All distances are in cm.

Lit	Sub	Sex	Diet	Group	NND mean	NND SE	Pair NND mean	Pair NND Indiv.	Co-occurence Freq	Co-occurence Pairs
39	A	M	F	7	4.13	1.81	32.31	41S	3	3
	B	M	F	12	4.06	2.13	33.69	41I	4	1
	E	F	W	8	2.75	1.11	22.75	39S	5	2
	F	F	F	7	5.25	5.65	21.44	39P	4	3
	K	F	F	7	7.41	8.93	20.69	39P	4	4
	N	M	F	7	3.07	1.86	16.13	41H	6	1
	O	F	F	12	3.94	4.93	25.81	39F	4	1
	P	M	W	8	6.31	8.20	20.69	39K	4	2
	R	F	W	8	5.06	2.98	21.63	39N	6	1
	S	F	F	9	7.69	10.25	22.75	39E	5	1
	T	M	F	9	2.44	0.68	16.56	41R	5	1
41	C	M	F	9	6.19	8.94	25.94	41L	3	5
	D	F	F	12	9.44	8.89	29.00	39E	3	3
	G	M	W	12	6.00	9.32	23.38	41M	6	1
	H	M	W	12	4.44	3.11	16.13	39N	5	1
	I	F	F	7	6.44	7.76	33.63	39B	3	2
	L	F	F	7	3.88	2.18	25.94	41C	3	2
	M	F	F	9	4.56	5.23	23.00	39P	6	1
	N	M	W	8	13.00	10.09	25.89	41R	1	8
	P	M	F	7	4.56	2.54	15.81	41S	4	3
	R	M	W	8	2.88	1.53	16.56	39T	5	4
	S	M	W	9	3.06	1.69	15.81	41P	4	2
	T	M	W	12	11.38	11.67	33.69	41D	2	1
	U	F	W	8	11.56	13.41	25.25	41P	4	2

Abbreviations: Sex- "M"=male, "F"=female; Diet- "F"=fish, "W"=worm; Pair NND "Indiv."= individual with smallest Pair NND; Co-occurrence- "Freq"= maximal number of readings found with another individual, "Pair"= number of animals forming pairs at the rate listed for "Freq".

Table 6.4: Description of the A) 8 strongest pairs of *T. butleri* generated by average linkage cluster analysis from Pair Nearest Neighbor Distance, and B) the two animals with the greatest average seperation distance (N = 276) observations, mean distance = 47.3 cm.).

Pair	Litter	Subject	Sex	Diet	Housing Group	Mean Distance	Shelters Shared
1	41	P	Male	Fish	7	15.8	4
	41	S	Male	Worm	9		
2	39	N	Female	Worm	8	16.1	5
	41	H	Male	Worm	12		
3	39	T	Male	Fish	9	16.6	5
	41	R	Male	Worm	8		
4	39	K	Female	Fish	7	20.7	3
	39	P	Male	Worm	8		
5	39	E	Female	Worm	8	22.8	5
	39	S	Female	Fish	9		
6	41	G	Male	Worm	12	23.4	6
	41	M	Female	Fish	9		
7	39	F	Female	Fish	7	25.8	3
	39	O	Female	Fish	12		
8	41	C	Male	Fish	9	25.9	2
	41	L	Female	Fish	7		
(B)	41	C	Male	Fish	9	89.6	3
	41	R	Male	Worm	8		

Note: Housing group "12" = individually housed.

Overall Nearest Neighbors (ONN)

Pairs of nearest neighbors across all readings were significantly biased toward those of the same litter and opposite diet as shown in table 6.4. This is true of both fish (baserate 0.43, trials 14, $p<0.007$), and worm-fed (baserate 0.61, trials 10, $p<0.003$), animals. ONN's were also significantly more likely to be unfamiliar in group 7 (baserate 0.65, trials 7, $p<0.0001$) and group 9 (baserate 0.76, trials 5, $p<0.0001$). The "pure" worm group (8), was the only group not to show a significant preference for unfamiliar animals, although the tendency was in that direction. Group "12," individually housed animals, were not familiar with any other snakes and were not considered in this comparison. There was no significant effect of sex. These results are summarized in Table 6.5.

Incidence of Co-occurrence

Direct Contact. The minimum number of readings that an animal (41N) was in physical contact with another was twice. One individual (41S) was found in direct contact with at least one other snake in all 8 readings. Snakes were in physical contact with another animal a mean of 5.5 readings.

It was found from direct contact information that only 7 pairs of snakes were ever in physical contact with each other. This occurred a maximum of 3 times across the

Table 6.5: Overall Nearest Neighbor comparisons of T. butleri in arena aggregation: Frequency that the individual with the least mean seperating 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	14	4	10 *
	Worm	10	1	9*
	(total)	24	5	19*
Litter:	39	11	7	4
	41	13	8	5
	(total)	24	15*	7
Sex:	Male	13	8	5
	Female	11	5	6
	(total)	24	13	11
Familiar			yes	no
Group:	7	7	0	7*
	8	6	1	5
	9	5	0	5*
	"12"	6	-	-
	(total)	24	1	17*

Note: "*" denotes $p<.05$, group "12" are individually housed.

8 readings. Only one of these pairs (39E & 39S) overlapped with those obtained by any other measures (i. e., ONN, NND).

Continuous mass. These data were intermediate in value between direct contact and same shelter pairing. Four pairs of snakes were found within the same continuous masses in 5 readings. All of these pairs were also noted in the same shelters data (below).

Same shelters. These data provided the most pairing information by generating 6 pairs with 5 co-occurrences each and 2 pairs with 6 co-occurrences each across the 8 readings. Since 55% of pairings occurred at a rate significantly above that of chance ($p<.01$), which were 2 or more pairings of the same individuals (as described in Table 6.6), the following comparisons were limited to only pairs of 5 or more co-occurrences, that is, those observed pairs with a .0001 chance of occurence (baserate 1/24 (0.0416), trials 8, 5 matches). It should also be noted that compared to the eight pairs generated by cluster analysis above, there was an overlap of four exact pairs of individuals, which gives some sense of convergent validity to this finding.

The eight most frequently occurring pairs as judged by shelter use (those with 63% to 75% co-occurrence) are shown in Table 6.7. These pairs consist of a) same diet: 2, opposite diet: 6, b) same sex: 4, opposite sex: 4,

Table 6.6: Table of binomial probabilities that snakes would co-occur under the same shelters across 8 readings in arena aggregation, and the frequencies of occurrence of each possibility.

Number of Readings Together	Frequency of Occurrence	Significance
0	52	0.288173
1	70	0.040994
2	76	0.003443
3	46	0.000183
4	22	0.000006
5	6	< 0.000001
6	2	< 0.000001
7	0	< 0.000001
8	0	< 0.000001

Note: 24 Snakes, 1/24 (0.0416), used as baserate and observations (8) as trials.

Table 6.7. Descriptions of the eight pairings of *T. butleri* found most frequently under the same shelter in arena aggregation.

Pair	Litter	Subject	Sex	Diet	Housing Group	Frequency of Shared Shelters
1	39	N	Male	Fish	7	6
	39	R	Female	Worm	8	
2	41	G	Male	Worm	12	6
	41	M	Female	Fish	9	
3	39	E	Female	Worm	8	5
	39	S	Female	Fish	9	
4	39	N	Male	Fish	7	5
	41	H	Male	Worm	12	
5	39	E	Female	Worm	8	5
	41	R	Male	Worm	8	
6	39	T	Male	Fish	9	5
	41	R	Male	Worm	8	
7	41	R	Male	Worm	8	5
	41	G	Male	Worm	12	
8	41	R	Male	Worm	8	5
	41	M	Female	Fish	9	

Note: Housing group "12" = individually housed.

c) same litter: 5, opposite litter: 3, and d) familiar: 1, unfamiliar: 7. Thus, these pairs consisted of unfamiliar animals on unfamiliar diets. It may be noted that five individuals occurred in more than one of these 8 pairings. Table 6.8a describes these "highly associating" animals, and compares them with (Table 6.8b) the 5 "least associating" snakes based on the maximum numbers of pairs they occur in.

Interestingly, two snakes were identified that rarely shared shelters with other individuals at all (see Table 6.8b). Snake 41N was found sharing shelters once each with only 8 other individuals. That leaves 15 other animals that this individual never co-occurred with, and it was never found under the highly popular shelter "G." In fact, this animal was found under no shelter more than twice, and was in the open on 3 of the 8 readings. Similarly, snake 39A shared shelters a maximum of 3 times with only 3 other snakes and never co-occurred at all with 9 others. Unlike 41N, this snake was always under cover, although it showed no site fidelity, being found under 5 different covers over the observations. These animals can be referred to then as "floaters," or even as "antisocial." Four other animals are also described in Table 6.8b based on their low maximal pairing rates. Of these animals, 41T shows a pattern similar to that of 41N: He is found in the open 3 times and under a different

Table 6.8: Description of individual T. butleri with A) very high (refer to Table 6.7) or B) low rates of association with other snakes in arena aggregation.

Litter Subject	Sex	Diet	Housing Group	Maximum Shared Shelters	Maximum Number of Pairs	Minimum Shared Shelters	Minimum Number of Pairs
A) High Association Rates							
41 R	M	W	8	5	4	0	2
39 E	F	W	8	5	2	0	2
39 N	M	F	7	6/5	1/1	0	4
41 G	M	W	12	6/5	1/1	0	2
41 M	F	F	9	6/1	1/1	0	2
39 S	F	F	9	5	1	0	1
39 T	M	F	9	5	1	0	1
41 H	M	W	12	5	1	0	4
B) Low Association Rates							
41 N	M	W	8	1	8	0	15
39 A	M	F	7	3	3	0	9
41 T	M	W	12	2	1	0	7
41 L	F	F	7	3	2	0	6
41 D	F	F	12	3	3	0	5
41 C	M	F	9	3	5	0	2

Notes: Shared Shelters = Frequency each subject was found under the same shelter as another specific individual; Number of Pairs = Number of individuals found occurring with given subject at the rate listed for Shared Shelters. (#/#: found more than once within top 8 but at different rates).

cover in the other 5 readings. 41L is more similar to 39A, only being in the open once but showing no site fidelity. 41D and 41C are both found on 3 readings under the "popular" shelter, G, thus showing site fidelity despite a relatively low pairing rate. These last two animals may demonstrate the transition between the "antisocial" animals and the more common "social" aggregators, since they show characteristics of both.

Comparison of Analyses

A comparison of the use of NNDs, ONNs, and COs described above is in order. Note that the basic results are closely paralleled in all tests although some details differ. These results are that specific pairs of closely associated animals were observed which generally consisted of unfamiliar littermates on different diets. In order to get a complete picture of occurrences all tests should be looked at 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 pairs without dual membership. In the comparison of co-occurrence, however, one individual may occur in multiple tightly associating pairs.

Similarly, the generation of "antisocials" differs in the two analyses. Taking the greatest sum distance between two individuals (as done in the cluster analysis) provides two animals which actually share shelters 3

times, one of which is part of one of the closely associated pairs. These are 41C and 41R, shown in Table 6.4b. Looking at freqency of co-occurrence we find animals that rarely share shelters with any other snakes, such as 41N and 39A (Table 6.8b), animals that also show a lack of site fidelity, one of which was often in the open and rarely in physical contact with other snakes. As such, frequency of co-occurance seems a much better demonstration of "antisociability."

Discussion

Thamnophis butleri is clearly an aggregating species based on the high frequency of co-occurrence under identical shelters in this test. This supports previous research, such as that by Noble and Clausen (1936). The sight of up to 15 out of 24 young snakes packed tightly under a single shelter (G) illustrates this well. Shelter "G" appears to demonstrate the kind of "habitat conditioning" Dundee and Miller (1968) speak of. This shelter attracted more snakes, repeatedly, than any other shelter, and was in no other way different from any other shelter in the arena.

What specifically attracted the snakes is a further question. When looking at the aggregation patterns of these animals, three different approaches (cluster analysis of Pair Nearest Neighbor Distances, comparison of

Overall Nearest Neighbor categories, and frequency of co-occurrence of pairs of animals under the same shelter) generated the same pattern: Specific pairs of animals could be identified which were more closely associated than others, and these pairs tended to consist of unfamiliar littermates on opposite diets.

The tendency of close pairs to be of differing diets parallels the findings in Chapter 5 that these snakes tended to prefer sites marked with feces based on the unfamiliar diet. There are several possible explanations if this is the case, such as reduction of competition for favored prey, or variation of diet for nutritionally-based reasons (see discussion in Chapter 4).

A preference to associate with littermates has been demonstrated in other taxa (Porter, McFadyen-Ketchum and King, 1989; Waldman and Bateson, 1989). Grouping with siblings may increase inclusive fitness for juveniles through benefits of aggregation as described above, such as a greater growth rate than individuals housed in isolation (Burghardt, in press). In adults, japanese quail have been shown to prefer to mate with individuals related at the level of "first cousin" (Bateson, 1982). Without knowing the relatedness of the two litters here, and given the possibility of multiple paternity as demonstrated by Schwartz (1989) for T. sirtalis, the reason for this attraction cannot be fully deciphered.

Similarly, associating with unfamiliar animals has arguable benefits as well. Since the first animals which could possibly become familiar are an individual, its mother, and its siblings, it could function to reduce competition between close relatives for resources, or to encourage dispersal of a litter over a greater area. This could in turn be beneficial for maximizing outbreeding as adults. These ideas were introduced previously as a possible account of the results seen in Chapter 5, site choice. The difficulty here is in the interactions of the apparent trends. Reasons to associate with kin seem to be mutually exclusive with many reasons to associate with unfamiliar animals.

It is also very interesting to have found specific individuals that seem to be avoiding aggregation rather than seeking out conspecifics like their counterparts. Subject 41N, specifically, showed no site fidelity, rarely shared shelters with the same snakes twice, and was seldom even found in physical contact with another snake. Further, this animal was never found under the highly popular shelter "G," and averaged greater distances from the other snakes than most other subjects. Why did this particular animal behave differently? It is possible that this avoidance of aggregations by a few individuals in a normally aggregating species is adaptively advantageous,

perhaps acting as a dispersal mechanism or as a "bet hedging" strategy.

In conclusion, T. butleri is a species that shows aggregative behavior and site fidelity. Pairs of animals found to associate most closely exhibited a consistent pattern of unfamiliar littermates on different diets. That snakes in a relatively homogenous environment show patterns of association corresponding to characteristics of other individual animals rather than the environment demonstrates that there is a social element to the aggregations 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. Based on those results a final consideration of possible interactions between key variables will be presented. The significance of these findings will occupy the remainder of this text.

Overview

As was outlined in the introduction, it is possible that given their high degree of reliance on chemical cues, snakes could be using conspecific feces as cues for aggregation. Further, such animals may be able to gather information regarding the diets of other animals in the area when choosing these sites.

It is known that neonate snakes show immediate preferences for certain prey, and that these preferences can be modified through experience. The research which is reported in this thesis was designed in part to consider the possibilty that these prey preferences may be reflected in aggregative behavior. In so doing, other possible attractants seen in other species, such as relatedness or familiarity, were also taken into consideration.

To investigate the importance of conspecific cues in aggregation behavior, an experimental program was designed in which neonate garter snakes were raised in group housing situations on diets of fish or earthworm, tested for prey preference, and subsequently site choice based on prey and conspecific cues. They were then observed in 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 Butler's garter snakes, Thamnophis butleri, born to 2 gravid females collected in 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 a preliminary study (Burghardt, in press; Burghardt and Lyman, in preparation), and for its tendency, although a worm specialist, to feed on fish in captivity (Halloy, 1987; Ruthven, 1908). It was believed that this would provide an interesting aspect to the dietary experience portion of these experiments as this species may have a very strong natural bias toward earthworms regardless of experience.

Execution of this program of research is what comprised this thesis. Chapter 2 discussed general methodology (origin of animals, housing, feeding, etc.). Chapter 3 concerned growth, and a variety of measures of

weight and length were made. Regarding the T. butleri in this study, it was clear that the main differences in mass, and in snout-vent length, was between the sexes; males being both heavier and longer than females throughout the study. The differences between measurements of growth show effects of litter, with litter 39 showing larger increases in weight, and litter 41 showing greater increases in length. Diet had only a small effect, with worm-fed snakes showing a greater percentage weight gain than those fed fish between the last two observations. Snakes also showed a general "slimming" of body shape over the course of the observations, as described by the Condition Index.

It was suggested that males are more capable of maintaining growth on less food than females, and that the litters may have allocated growth in different ways. The possible effects of handling stress and food limitations were also discussed. Most importantly for the present research, the two diets showed very little differences in animal size or growth. 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.

Prior to starting this work several hypotheses were tenatively formed, the results of which will be discussed here in review:

(1) Regarding chemical preference, it was hypothesized that the initial response would be to favor the natural diet of earthworms. And, (2) since this species is an earthworm specialist, one would expect the responses to chemical prey cues after experience with prey to also favor earthworms, though the animals fed fish should show a higher response to that prey than inexperienced snakes.

Prior to feeding experience, the T. butleri in this experiment had shown a clear preference for earthworm extract over fish extract or a water control, as was expected given their natural prey. Subsequent experience with prey resulted in a dramatic increase in responsiveness to fish extract in those animals fed fish, whereas snakes on the worm diet showed no change in responses between tests. The final result was that T. butleri that had never fed upon earthworms maintained a fairly high response to that prey, while those naive to fish never aquired much interest in those cues. A high response to fish came only through experience with that prey.

Other hypotheses were: (3) Site choice should be in favor of the preferred prey cue, probably earthworm. This

would make ecological sense, since prey should be more likely to be found where its chemical cues are strongest. And, (4) Site choice based on feces should also be in favor of cues based on the preferred prey. If the animals prefer to be in association with other snakes feeding on the same preferred prey, then perhaps the reason is to find areas rich in that resource.

Having established prey preferences through extract testing, Chapter 5 examined the possibility that information transfer concerning diet could be detected in a site choice test. In these experiments the role of prey cues in habitat choice were assessed for the Thamnophis butleri subjects. These animals were tested using both direct prey cues (provided by aqueous chemical extracts prepared from prey surfaces) and conspecific feces. In the Initial Feces-Based Choice Test, the animals were shown to have an overall bias in favor of feces carrying worm cues. This was not surprising given their natural diet being earthworms. However, in the Final Feces-Based Choice Test, it was found that dietary experience had an effect on site choice such that snakes spent more time associated with the feces derived from the unfamiliar diet. 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. It was also noted that these same animals that had shifted

site preference based on feces were more likely to be unaffected by external cues in this choice.

The finding that the test animals failed to show preferences toward any site based on prey surface cues was surprising, and is assumed to be related to the predatory specialization of this species. However, if it is true that cues based on prey extracts are not utilized in habitat choice by T. butleri, then it is possible that it is not prey information that is of interest in choices based on conspecific feces. If these choices were motivated by some factor such as interest in an unfamiliar diet based on nutritive value, etc., it seems likely that it would be reflected in choices based on the extracts of the prey as well. Earlier results regarding growth implied there were no such differences.

This leads to the possibility that familiarity may be the factor in question regarding site choice. Most snakes fed on one diet or the other were housed with other snakes fed the same thing, therefore familiar feces would carry cues of the same diet. It is possible that the prey information contained in feces became a marker identifying familiar animals. Since the preferences shown were for feces based on the unfamiliar diet, these snakes may be attracted to unfamiliar conspecifics. Such an attraction allows for a variety of interesting possibilities, although why such a tendency was shown by animals fed on

worms so much more than by those fed fish is perplexing. It is possible that the stronger attraction to fish feces cues is due to novelty; an animal eating something that unusual must be unfamiliar. Meanwhile, animals feeding on fish still have a natural "expectation" for conspecific feces to be carrying worm cues, decreasing the significance of the occurrence.

Most important, it was hypothesized, (5) that animals would aggregate preferentially with other animals feeding on the same, preferred, diet. This would indicate the use of information transfer via feces cues to locate areas rich in preferred prey based on information gained from other snakes in the area.

The possibilities suggested by the habitat choice tests make the results of Chapter 6 all the more interesting with respect to this hypothesis. In this chapter the T. butleri housed with conspecifics or alone were observed for aggregative behavior in a single large arena. The animals readily aggregated under preferred shelters, as expected from previous work (Dundee and Miller, 1968; Noble and Clausen, 1936). The pattern seen across several methods of comparison was the intriguing part: Specific pairs of animals could be identified which were more closely associated than others, and these pairs tended to consist of unfamiliar littermates on opposite diets.

Interactions

Comparisons between experiments have been occasionally pointed out over the course of this thesis. The first was the coincidence between growth in weight and responsiveness to chemical prey extracts of animals housed alone or with T. sirtalis. In this case, snakes housed in isolation grew more and had lower responses to chemical cues than any other groups, while those housed with T. sirtalis had the lowest growth and highest responsiveness to chemical cues of all the subjects. The next comparison was between the results of chemical prey preference tests and those of site choice tests. Animals tended to show higher responses toward the chemical cues from the prey they were familiar with, yet preferences for feces cues were more likely for the unfamiliar prey. Lastly, there was a parallel between this choice of sites marked with feces bearing unfamiliar prey cues and the likelihood of close pairs in aggregation to be unfamiliar animals on unfamiliar diets.

In all, this is painting a very complex and intriguing picture of the aggregation preferences of these young garter snakes. It is desirable to further these comparisons in search of any other clues towards the deciphering of this behavior. To accomplish this, variables reflecting each factor tested here were be analyzed for correlations across experiments. In this way

any relationships between such factors as growth rate, chemical response and habitat choice may be outlined, and interactions of chemical preference and habitat choice reviewed. The final comparison will be to compare all of these factors to the aggregation patterns seen in the last experiment.

Methods

Measures

When at all possible, measures were chosen that could reflect responses for each test with a single value per subject. Therefore, 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.

Aspects of growth measured here were not as clear-cut, since there were different effects whether one looked at absolute size or percent increase. For this analysis, the initial and final value of snout-vent length (SVL1, SVL3) and mass (Mass1, Mass2) were both included, along with percent snout-vent (PSV) and mass (PMI) increase. Similarly, there was no one dependent measure for aggregation behavior, so the mean distance to each

subjects' single nearest neighbor across all trials (NND) was used.

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 seperated by diet as well as pooled. NND was only utilized in the test using aggregation tested animals. Correlations were run using Statgraphics 3.0 (STSC, 1988).

Subjects were also rank-ordered with regard to each variable to allow for direct comparison of individual subjects such as those that were frequent aggregators or "antisocial."

Results

Pearson correlation analysis suggests some connections between factors that had not been noticeable before. Correlation coefficients are summarized in Table 7.1 for the analysis using all subjects. R values with a significance of $p<0.10$ (or smaller) are reported for both diet groups and all snakes. Results for Prey Surface-Based Site Choice, and percent size increase (PSV and PMI) are not included in this table due to a lack of any significant correlations with other variables.

Note that in addition to being correlated with other measures of size, final snout-vent lenght (SVL3) was significantly negatively correlated ($R = -.53$, $p<0.05$) with initial feces-based site choice (Feces Choice1), such that animals with stronger initial responses to worm-based feces ended up being longer animals. Also of particular interest is that the initial TFAS response to worm was significantly correlated with several variables, including a negative correlation with size for fish-eaters, and initial feces-based site choice for worm fed animals.

Table 7.2 similarly summarizes Pearson correlation coefficients of the analyses using aggregation tested animals. In this case, note that the fish fed snakes showed significant correlations between nearest neighbor distance (NND) and both initial snout-vent length (SVL1) and final mass (Mass3). This implies that larger animals showed greater distances between nearest neighbors. Initial mass (Mass1) was also significantly correlated with final feces-based site choice in worm fed animals. Chemical preference values correlated in a similar pattern to that seen in the analysis of all animals, with the addition of a significant correlations between final fish - worm chemical preference scores and initial feces-based site choice.

Comparing the ranks of the 5 most frequently paired subjects reveals only one trend not predictably associated

Table 7.1: Pearson correlation coefficients of $p < 0.1$ among variables across experiments, using all *T. butleri* subjects (N=38).

Var.	diet	Worm1	Fish1	F-W1	Worm2	Fish2	F-W2	Feces Choice1	Feces Choice2	Mass1	Mass3	SVL1	SVL3
Fish1	all	-											
	fish	.458+											
	worm	-											
F-W1	all	-	.290										
	fish	.443	-										
	worm	-	-										
Worm2	all	.439*	-	-									
	fish	-	-	-									
	worm	.589*	-	-									
Fish2	all	.342+	-	.343+	.378+								
	fish	.508+	-	-	.681*								
	worm	-	-	.490+	-								
F-W2	all	-	-	-	-	-							
	fish	.452	-	.558+	-	-							
	worm	-	-	-	-	-.446							
Feces Choice1	all	.399+	-	-	-	-	-						
	fish	-	-	-	-	-	-						
	worm	.623*	-	-	-	-	-						
Feces Choice2	all	-	-	-	-	-	-	-					
	fish	-	-	-	-	-	-	-					
	worm	-	-	-	-	-	-	-					
Mass1	all	-.457*	-	-	-	-	-.293	-	-				
	fish	-.679*	-	-	-	-	-	-	-				
	worm	-	-	-	-	-	-	-	.424				
Mass3	all	-	-	-	-	-	-	-	-	-			
	fish	-.552+	-	-	-	-	-	-.437	-	.586*			
	worm	-	-	-	-	-	-	-	-	-			
SVL1	all	-	-	-	-	-	-	-	-	.695**	.292		
	fish	-.558+	-	-	-	-	-	-	-	.756**	.767**		
	worm	-	-	-	-	-	-	.446	-	.491+	.397		
SVL3	all	-.397+	-	-	-	-	-	-	-	.703*	.296	.749**	
	fish	-.691*	-	-	-	-	-	-.531+	-	.738**	.849**	.781**	
	worm	-	-	-	-	-	-	-	-	.649*	.391	.696**	

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, Mass1=initial mass, Mass3=final mass, SVL1=initial snout-vent length, SVL3=final snout-vent length.

Table 7.2. Pearson correlation coefficients of $p < 0.1$ among variables across experiments, using only aggregation-tested T. butleri subjects (N=24).

Var.	diet	Worm1	Fish1	F-W1	Worm2	Fish2	F-W2	Feces Choice1	Feces Choice2	Mass1	Mass3	SVL1	SVL3
Fish1	all	-											
	fish	-											
	worm	-											
F-W1	all	-	-										
	fish	.549	-										
	worm	-	-										
Worm2	all	.623*	-	-									
	fish	.546	.620+	.569									
	worm	.695*	-	-									
Fish2	all	.443+	-	-	.406+								
	fish	-	.567	-	.741*								
	worm	-	-	-	-								
F-W2	all	-	-	-	-	-							
	fish	.694+	-	.801*	.553	-							
	worm	-.514	-	-	-	-.568							
Feces Choice1	all	.384	-	-	-	-	-.503+						
	fish	-	-	-	-	-	-.535						
	worm	.627+	-	-	-	.526	-.611+						
Feces Choice2	all	.397	-	-	-	-	-	-					
	fish	-	-	-	-	-	.566	-					
	worm	-	-	-	.486	-	-	-					
Mass1	all	-.347	-	-	-	-	-	-	-				
	fish	-.699+	-	-	-	-	-	-	-				
	worm	-	-	-	-	-	-	-	.650+				
Mass3	all	-	-	-	-	-	-	-	-	-			
	fish	-	-	-	-	-	-	-	-	.568			
	worm	-	-	-	-	-	-	-	-	-			
SVL1	all	-	-	-	-	-	-	-	-	.639**	.347		
	fish	-.561	-	-	-	-	-	-	-	.817*	.738*		
	worm	.529	-	-	-	-	-	.520	-	-	.513		
SVL3	all	-	-	-	-	-	-	-	-	.588*	.412+	.745**	
	fish	-.624+	-	-	-	-	-	-.574	-	.662+	.911**	.793*	
	worm	-	-	-	-	-	-	-	-	-	.759*	.682+	
NND	all	-	-	-	-	-	-	-	-	-	.374	-	-
	fish	-	-	-	-	-	-	-	-	-	-	-	-
	worm	.483	-	-	-	-	-	-	-	-	.558+	.589+	-

Note: + = $p<0.05$, * = $p<0.01$, ** = $p<0.001$.
Abbreviations: NND=nearest neighbor distance, being the smallest mean separating distance between individuals across 8 readings. Others as in Table 7.1.

with their already-shown predilictions regarding diet, etc. This is the change in SVL ranking among snakes. All of these 5 snakes increased in SVL relative to the other snakes. The subjects most likely to associate with these animals showed no pattern. Similarly, the 5 animals with the least NND all showed increased rank position in either mass or SVL. It should be noted that the most "antisocial" snake, 41N, also increased in SVL rank, although 3 of the 6 snakes with the greatest NNDs fell in rank. The mean PSV and PWT were both higher for the aforementioned "highly social" subjects (0.170 and 0.181, respectively) than for these 6 "unsocial" snakes (0.158 and 0.149 respectively). These comparisons are summarized in Table 7.3.

Final Discussion

Snakes have often been considered the least social of reptiles, themselves long thought to lack complex behaviors. However, a variety of social behaviors have been documented in snakes, including male combat, courtship, mating aggregations, communal nesting, and hibernation aggregations. Aggregations outside of reproductive and hibernation periods also occur, but the reasons are not always so clear. The results of this thesis are intended to shed some light on the cues and reasons involved in this little understood behavior.

Table 7.3: Descriptions of A) most tightly grouped and B) least tightly grouped subjects regarding rank size and percent size increase.

Subject	SVL1	SVL3	PSV	Mass1	Mass3	PMI	Pair	NND
A) Tightly grouped								
39N	9	5	.180	5	12	.109	6	2
41M	35	30	.022	18	34	-.114	6	8
41G	15	2	.232	3	2	.345	6	9
41R	7	1	.202	8	7	.194	5	3
39E	26	21	.165	26	20	.163	5	7
41H	25	7	.213	10	4	.198	5	2
39K	11	11	.157	21	21	.115	4	4
39P	5	17	.108	20	8	.297	4	4
41P	33	9	.276	25	24	.373	4	1
41S	22	26	.148	34	16	.373	4	1
		Mean:	.170		Mean:	.181		
B) Least grouped								
41N	31	29	.169	14	17	.085	1	12
41T	1	1	.152	4	10	.030	2	15
39A	8	8	.147	22	11	.250	3	14
41D	38	38	.154	38	38	.264	3	14
41I	37	35	.200	37	35	.242	3	15
39B	29	33	.125	30	33	.025	4	15
		Mean:	.158		Mean:	.149		

Abbreviations: SVL1, SVL3= initial and final ranked snout-vent length; PSV=percent snout-vent increase; Mass1, Mass3= initial and final ranked mass; PMI=percent mass increase; Pair=maximum number of readings (of 8) sharing shelters with another individual; NND=ranked nearest neighbor distance.

It was hypothesized that snakes may be engaging in information transfer in aggregations, gaining information regarding foraging sites or food availabilty. The attraction of many snakes to conspecific feces seemed to support this idea. The current research has shown that T. butleri are capable of using feces cues to choose habitats, and that these choices are predictable based on dietary experience. However, evidence has been building that the primary attractant is actually unfamiliarity rather than diet per se.

In the investigation of interactions between experiments undertaken above, some correlations occur that should be underscored despite being nonsignificant. The interactions of initial mass with final chemical prey preference tests seem to relate to the initial tendency of smaller animals to accept fish. It had been noted that smaller snakes had been among those accepting fish when diets were first being assigned, and these animals would then have increased responsiveness to fish through experience. At the time this was barely noticeable, and not at all significant, yet as time went on and experiential effects added together, nearly significant correlations occurred.

This at the very least demonstrates the importance of carefully balancing subjects for all variables at the onset of subsequently related experiments. It also raises

the question of whether fish may be recognized as a better "growth" food for initially small animals. Scudder-Davis and Burghardt (1987) had shown increased growth rates in T. radix fed fish over those fed worms when no mineral supplementation was used. It has already been argued that T. butleri may retain responses to fish chemical cues from their evolution from T. radix, and it seems likely that the same phenomenon may be occurring here. Fish are not preyed upon in the wild by this species, but ancestral tendencies may effect greater responsiveness to this prey in individuals smaller at birth, who would benefit from greater growth rates than their larger counterparts.

The most interesting interaction here is that between snake size and aggregating behavior. The correlation between these variables is only significant for animals on the worm diet, but the implication is that more closely grouping snakes tended to be relatively large. And it is the case that all of the most frequently pairing animals showed an increase in rank size--that is, they consistently out-grew other snakes such that their rank position SVL and/or mass increased between the initial and final observations, regardless of absolute size. The greater mean percent increase in size of these animals compared to the 6 least tightly grouping snakes agrees with this trend. Which direction this may be acting is unclear, however. Are the animals growing better than

their counterparts due to some benefit gained by their being highly aggregative, or are other animals attracted to them because they are growing? Since no information was gathered here regarding order of shelter occupation, trailing behavior, or individual interaction, this is not clear. Likewise, those least-tightly grouping subjects who did not increase in size as well could either be avoiding others or being avoided.

However, the presence, and comparable growth, of snakes such as the "antisocial" 41N may be a clue. This animal appears to have avoided conspecifics, as demonstrated by its never being found under the popular shelter "G," and its frequent appearance in the open. Since no other animals seem to have been attracted to this snake despite its growth, this may be evidence that the highly social snakes were also determining their own social patterns. Since both cases resulted in growth, it appears that alternative strategies may carry similar growth benefits. What differed between this extreme animal and other snakes with greater NNDs is at this point unclear.

In the introduction the definition of the term "aggregation" was discussed, and whether it carried social implications or not. For this thesis 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. In these experiments it has been demonstrated that neonate T. butleri are attracted to conspecifics and their cues, and that this attraction is based on characteristics of other individual animals. This implies a non-random basis to this behavior: sites were chosen based on the occurrence of other snakes there, and more importantly, other snakes with particular characteristics. Such results establish the plausibility of something like information transfer in these reptiles. If the attraction were solely for improved environmental conditions afforded by clustered animals, there should have been no patterns among the animals that were most likely to be together. That characteristics of particular animals is important in aggregation seems to demonstrate a possibly "true" social behavior.

These experiments have also shown that much is still in need of clarification. There is a need to further investigate the interaction of familiarity and diet in aggregation behavior. Experiments must be performed using situations in which these factors can be better controlled than in previous studies. A larger number of animals such that there are both familiar and unfamiliar individuals available on each diet would be requisite for such research.

The cues that can be carried in integument should also be investigated. Earlier work (Graves and Halpern, 1988) demonstrated attraction of garter snakes to conspecific skin extracts. Can this cue carry the same type of information as feces? 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.

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? The occurrance and importance of "antisocial" animals of an aggregating species also requires further investigation.

In all, at least as many questions have been raised by the research described here as have been answered. Thamnophis butleri do show effects of dietary experience on prey preferences, but these do not affect site choice based on prey cues. These snakes do, however, choose sites based on conspecific feces cues that may carry information regarding familiarity as well as dietary cues. Thamnophis butleri neonates also show aggregative

behaviors with patterns of individual characteristics, which implies social factors in attraction and not just habitat choice based on mere environmental conditions. However, the exact attractant to these aggregations is still uncertain, as are possible dynamics among individuals and their relationship to the natural environment of this species.

LITERATURE CITED

Allee, W. C. 1931. Animal aggregations. University of Chicago Press, Chicago.

Allee, W. C. 1938. The social life of animals. Beacon Press, Beacon Hill, Boston.

Allen, B., Burghardt, G. M., and York, D. S. 1984. Species and sex differences in substrate preferences and tongue flick rate in three sympatric species of water snakes (Nerodia). J. Comp. Psych. 98: 358-367.

Altmann, S. A. and Altmann, J. 1970. Baboon ecology: African field research. University of Chicago Press, Chicago.

Andrews, R. M. 1982. Patterns of growth in reptiles. In C. Gans and F. H. Pough (Eds.), Biology of the reptilia, Vol. 13, pp. 273-334. Academic Press, New York.

Arnold, S. J. 1977. Polymorphism and geographic variation in the feeding behavior of the garter snake, Thamnophis elegans. Science 197: 676-678.

Arnold, S. J. 1978. Some effects of early experience on feeding responses in the common garter snake, Thamnophis sirtalis. Anim. Behav. 26:455-462.

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

Arnold, S. J. and Wassersug, R. J. 1978. Differential predation on metamorphic anurans by garter snakes (Thamnophis): social behavior as a possible defense. Ecology 59: 1014-1022.

Barbour, R. W. 1962. An aggregation of copperheads, Agkistrodon contortrix. Copeia 1962: 640.

Barker, D. G., Murphy, J. B., and Smith, K. W. 1979. Social behavior in a captive group of Indian pythons, Python molurus (Serpentes, boidae) with formation of a linear social hierarchy. Copeia 1979: 466-471.

Bateson, P. 1982. Preferences for cousins in Japanese quail. Nature 295: 236-237.

Blair, W. F. 1960. The rusty lizard: A population study. University of Texas Press, Austin.

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

Burghardt, G. M. and Denny, D. 1983. Effects of prey movement and prey odor on feeding in garter snakes. Z. Tierpsychol. 62: 329-347.

Burghardt, G. M. and Pruitt, C. H. 1975. Role of the tongue and senses in feeding of naive and experienced garter snakes. Physiol. Behav. 14: 185-194.

Burghardt, G. M. and Rand, A. S. 1985. Group size and growth rate in hatchling green iguanas (Iguana iguana). Behav. Ecol. Sociobiol. 18: 101-104.

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

Carpenter, C. C. 1952b. Growth and maturity of the three species of Thamnophis in Michigan. Copeia 1952: 237-243.

Carpenter, C. C. 1977. Communication and displays of snakes. Amer. Zool. 17: 217-223.

Carr, C. M. and Gregory, P. T. 1976. Can tongue flicks be used to measure niche sizes? Can. J. Zool. 54: 1389-1394.

Chiszar, D. and Carter, T. 1975. Reliability of individual differences between garter snakes (Thamnophis radix) during repeated exposures to an open field. Bull. Psychon. Soc. 5: 507-509.

Chiszar, D. and Radcliffe, C. W. 1976. Rate of tongue flicking by rattlesnakes during successive stages of feeding on rodent prey. Bull. Psychon. Soc. 7: 485-486.

Chiszar, D., Scudder, K., and Knight, L. 1976. Rate of tongue flicking by garter snakes (Thamnophis radix haydeni) and rattlesnakes (Crotalus v. viridis, Sistrurus catenatus tergeminus, and Sistrurus catenatus edwardsi) during prolonged exposure to food odors. Behav. Biol. 18: 273-283.

Chiszar, D., Wellborn, S., Wand, M. A., Scudder, K. M., and Smith, H. M. 1980. Investigatory behavior in snakes, II: Cage cleaning and the induction of defecation in snakes. Anim. Learn. Behav. 8: 505-510.

Conant, R. 1950. On the taxonomic status of Thamnophis butleri (Cope). Bull. Chic. Acad. Sciences 9: 71-77.

Conant, R. 1975. A field guide to reptiles and amphibians of eastern and central North America. Houghton Mifflin Co., Boston.

Cooper, W. E. Jr., and Burghardt, G. M. 1990. A comparative analysis of scoring methods for chemical discrimination of prey by squamate reptiles. J. Chem. Ecol. 16: 45-65.

Crews, D. and Garstka, W. R. 1982. The ecological physiology of a garter snake. Sci. Am. 247: 159-168.

Crews, D., Diamond, M. A., Whittier, J., and Mason, R. 1985. Small male body size in garter snake depends on testes. Am. J. Physiol. 249 (Regulatory Integrative Comp. Physiol. 18): R62-R66.

Davis, D. D. 1932. Occurrence of Thamnophis butleri Cope in Wisconsin. Copeia 1932: 113-118.

Drickamer, L. C. 1989. Odor preferences of wild stock female house mice (Mus domesticus) tested at three ages using urine and other cues from conspecific males and females. J. Chem. Ecol. 15: 1971-1987.

Dundee, H.A. and Miller III, M. C. 1968. Aggregative behavior and habitat conditioning by the prairie ringneck snake, Diadophis punctatus arnyi. Tul. St. Zool. Bot. 15: 41-58.

Duvall, D., King, M. B., and Gutzwiller, K. J. 1985. Behavioral ecology and ethology of the prairie rattlesnake. Nat. Geogr. Res. 1: 80-111.

Emlen, S. T. 1980. Ecological determination and sociobiology. In G. W. Barlow and J. Silverberg (Eds.), Sociobiology: Beyond nature/nurture? Westview Press, Inc., Boulder, Colo.

Finneran, L. C. 1949. A sexual aggregation of the garter snake Thamnophis butleri (Cope). Copeia 1949: 141-144.

Fuchs, J. L. and Burghardt, G. M. 1971. Effects of early feeding experience on the responses of garter snakes to food chemicals. Learn. Mot. 2: 271-279.

Galef, B. G. Jr. 1986. Olfactory communication among rats: Information concerning distant diets. In D. Duvall, D. Muller-Schwartze, and R. M. Silverstein (Eds.), Chemical signals in vertebrates, vol. 4, pp. 487-505. Plenum Press, New York.

Galef, B. G. Jr. 1988. Communication of information concerning distant diets in a social, central-place foraging species: Rattus norvegicus. In T. R. Zentall and B. G. Galef, Jr. (Eds.) Social learning, pp.119-140. Lawrence Erlbaum, Hillsdale, New Jersey.

Gillingham, J. C. 1987. Social behavior. In R. A. Seigel, J. T. Collins, and S. S. Novak (Eds.), Snakes: Ecology and evolutionary biology, pp. 184-209. Macmillan, New York.

Gillingham, J. C., Rowe, J., and Weins, M. A. in press. Chemosensory orientation and earthworm location by foraging eastern garter snakes, Thamnophis s. sirtalis. In D. McDonald, D. Muller-Schwarze, and S. Natynczuk (Eds.) Chemical signals in vertebrates, vol. 5. Oxford University Press, Oxford.

Godley, J. S. 1980. Foraging ecology of the striped swamp snake, Regina alleni, in southern Florida. Ecol. Monogr. 50: 411-436.

Golan, L., Radcliffe, C., Miller, T., O'Connell, B., and Chiszar, D. 1982. Trailing behavior in prarie rattlesnakes (Crotalus viridis). J. Herpet. 16: 287-293.

Gove, D. and Burghardt, G. M. 1983. Context-correlated parameters of snake and lizard tongue-flicking. Anim. Behav. 31: 718-723.

Graves, B. M. and Halpern, M. 1988. Neonate plains garter snakes (Thamnophis radix) are attracted to conspecific skin extracts. J.Comp. Psych. 102: 251-253.

Graves, B. M., Duvall, D., King, M. B., LIndstedt, S. L., and Gern, W. A. 1986. Initial den location by neonatal prairie rattlesnakes: Functions, causes, and natural history in chemical ecology. In D. Duvall, D. Muller-Schwarze, and R. M. Silverstein (Eds.) Chemical signals in vertebrates, vol. 4, pp. 285-304. Plenum, New York.

Gregory, P. T. 1975. Aggregations of gravid snakes in Manitoba, Canada. Copeia 1975: 185-186.

Halloy de Grosse, M. 1987. A comparative study of the ontogeny of prey ingestion in four species of Thamnophis (Serpentes: Colubridae). Master's thesis, University of Tennessee, Knoxville.

Halpern, M. 1987. Vomeronasal system functions: Role in mediating the reinforcing properties of chemical stimuli. In W. K. Schwerdtfeger and W. J. A. J. Smeets (Eds.) The foregreain of reptiles, pp. 142-150. Karger, New York.

Halpern, M. and Kubie, J. L. 1983. Snake tongue flicking behavior: Clues to vomeronasal system functions. In D. Muller-Schwarze and R. M. Silverstein (Eds.), Chemical signals in vertebrates, pp. 45-72. Plenum, New York.

Halpin, Z. T. and Hoffman, M. D. 1987. Sibling recognition in the white-footed mouse, Peromyscus leucopus: association or phenotype matching? Anim. Behav. 35: 563-570.

Hamilton, W. D. 1971. Geometry for the selfish herd. J. Theor. Biol. 31: 295-311.

Heller, S. B., and Halpern, M. 1982a. Laboratory observations of aggregative behavior of garter snakes, Thamnophis sirtalis. J. Comp. Physiol. Psych. 96:967-983.

Heller, S. B., and Halpern, M. 1982b. Laboratory observations of aggregative behavior of garter snakes, Thamnophis sirtalis: roles of the visual, olfactory, and vomeronasal senses. J. Comp. Physiol. Psych. 96:984-999.

Horne, E. A. and Jaeger, R. G. 1988. Territorial pheromones of female red-backed salamanders. Ethology 78: 143-152.

Immelmann, K. and Beer, C. 1989. A dictionary of ethology. Harvard University Press, Cambridge.

Jackson, C. G. Jr. 1970. A biometrical study of growth in Pseudemys concinna suwanniensis. Copeia 1970: 528-534.

Jaeger, R. G. 1981. Dear enemy recognition and the costs of aggression between salamanders. Amer. Natur. 117: 962-974.

Jaeger, R. G. 1986. Pheromonal markers as territorial advertisement by terrestrial salamanders. In D. Duvall, D. Muller-Schwarze, and R. M. Silverstein (Eds.) Chemical signals in vertebrates, vol. 4, pp. 191-203. Plenum, New York.

Jaeger, R. G., Goy, J. M., Tarver, M., and Marquez, C. E. 1986. Salamander territoriality: pheromonal markers as advertisement by males. Anim. Behav. 34: 860-864.

Kauffeld, C. F. 1953. Methods of feeding captive snakes. Herpetologica 9: 129-131.

Kephart, D. G. 1982. Microgeographic variation in the diets of garter snakes. Oecologia 52: 287-291.

King, M., McCarron, D., Duvall, D., Baxter, G., and Gern, W. 1983. Group avoidance of conspecific but not interspecific chemical cues by prairie rattlesnakes (Crotalus viridis). J. Herp. 17: 196-198.

Kropach, C. 1971. Sea snake (Pelamis platurus) aggregations on slicks in Panama. Herpetologica 27: 131-135.

Kubie, J. L. 1977. The role of the vomeronasal organ in garter snake prey trailing and courtship. Doctoral dissertation, Downstate Medical Center, State University of New York.

Kubie, J. and Halpern, M. 1975. Laboratory observations of trailing behavior in garter snakes. J. Comp. Physiol. Psych. 89: 667-674.

Kubie, J. and Halpern, M. 1978. Garter snake trailing behavior: Effects of varying prey-extract concentration and mode of prey-extract presentation. J. Comp. Physiol. Psych. 92: 362-373.

Myers, B. C. and Eells, M. A. 1968. Thermal aggregation in Boa constrictor. Herpetologica 24: 61-66.

Noble, G. K. and Clausen, H. J. 1936. The aggregation behavior of Storeria dekayi and other snakes, with especial reference to the sense organs involved. Ecol. Monog. 6: 271-316.

Overmann, S. R. 1970. Social facilitation of feeding in box turtles and American toads. Amer. Zool. 10: 473 (abstract).

Porter, K. R. 1972. Herpetology. Saunders, Philadelphia.

Porter, R. H. and Czaplicki, J. A. 1974. Responses of water snakes (Natrix r. rhombifera) and garter snakes (Thamnophis sirtalis) to chemical cues. Anim. Learn. Behav. 2: 129-132.

Porter, R. H., Matochik, J. A., and Makin, J. W. 1986. Discrimination between full-sibling spiny mice (Acomys cahirinus) by olfactory signatures. Anim. Behav. 34: 1182-1188.

Porter, R. H., Sentell, S. W., and Makin, J. W. 1987. Effects of intranasal ZnSO4 irrigation are mitigated by the presence of untreated littermates. Pysiol. Behav. 40: 97-102.

Porter, R. H., McFadyen-Ketchum, S. A., and King, G. A. 1989. Underlying bases of recognition signatures in spiny mice, Acomys cahirinus. Anim. Behav. 37: 638-644.

Prater, S. H. 1933. The social life of snakes. J. Bombay Nat. Hist. Soc. 36: 469-476.

Ruthven, A. G. 1908. Variations and genetic relationships of the garter-snakes. Bull. U. S. Nat. Museum 61: xii-201.

SAS 1985. SAS, version 5. SAS Institute, Inc., Cary, N.C.

Schell, F. M. and Weldon, P. J. 1985. ^{13}C-NMR analysis of snake skin lipids. Agric. Biol. Chem. 49: 3597-3600.

Schmidt, K. P. 1938. Herpetological evidence for the postglacial eastward extension of the steppe in North America. Ecology 19: 396-407.

Schwartz, J. M. 1989. Multiple paternity and offspring variability in wild populations of the garter snake, Thamnophis sirtalis (Colubridae). Doctoral dissertation, University of Tennessee at Knoxville.

Scott, J. P. 1956. The analysis of social organization in animals. Ecology 37: 213-221.

Scudder, K. M., Stewart, N. J., and Smith, H. M. 1980. Response of neonate water snaks (Nerodia sipedon) to conspecific chemical cues. J. Herpet. 14: 196-198.

Scudder, R. M. 1983. Food consumption and growth in three species of water snakes of the genus Nerodia (Colubridae). Doctoral dissertation, University of Tennessee, Knoxville.

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

Semlitsch, R. D. and Gibbons, R. D. 1982. Body size dimorphism and sexual selection in two species of water snakes. Copeia 1982: 974-976.

Sheffield, L. P., Law, J. H., and Burghardt, G. M. 1968. On the nature of chemical food sign stimuli for newborn snakes. Comm. in Behav. Biol. 2: 7-12.

Siegel, S. 1956. Nonparametric statistics for the behavioral sciences. McGraw Hill, New York.

Sokal, R. R. and Michener, C. D. 1958. A statistical method for evaluating systematic relationships. Univ. Kansas Sci. Bull. 38: 1409-1438.

STSC 1988. Statgraphics, version 3.0. STSC, Inc., Rockville, MD.

Swain, T. A. and Smith, H. M. 1978. Communal nesting in Coluber constrictor in Colorado (Reptilia: Serpentes). Herpetologica 34: 175-177.

Tinbergen, N. 1953. Social behavior in animals. Wiley and Sons, Inc., New York.

Torrey, T. W. 1971. Morphogenesis of the vertebrates, third ed. John Wiley and Sons, Inc., New York.

Waldman, B. and Bateson, P. 1989. Kin association in Japanese quail chicks. Ethology 80: 283-291.

Ward, P. and Zahavi, A. 1973. The importance of certain assemblages of birds as "information-centres" for food-finding. Ibis 115: 517-534.

Wilde, W. S. 1938. The role of Jacobson's organ in the feeding reaction of the common garter snake, Thamnophis sirtalis sirtalis (Linn.). J. Exper. Zool. 77: 445-465.

Wilson, E. O. 1975. Sociobiology. Belknap/Harvard University Press, Cambridge.

Zajonc, R. B. 1971. Attraction, affiliation and attachment. In J. F. Eisenberg and W. S. Dillon (Eds.) Man and beast: Comparative social behavior. Smithsonian Institution Press, Washington, D. C.

VITA

Lani Patricia Lyman 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 Master of Sciences degree in Life Sciences: Ethology in 1990.

While in Tennessee, Lani has acquired a great many prized creatures, namely among them one Tracy Branson Henley. In addition she also has collected 8 aquaria full of mostly tropical fish, several lizards, snapping turtles, one salamander, a caecilian, Madagascaran Hissing Cockroaches, and, of course, several species of snake.

Lani Lyman intends to continue her studies and complete a PhD dissertation here at Tennessee. She also hopes to find some time to resume her neglected artistic pursuits, breed snakes of interesting colors, relax, and keep a real good hold on that Henley fellow.