Seasonal trailing behavior and corticosterone levels in male red-sided garter snakes (Thamnophis sirtalis parietalis)

Pamela Kay Thinesen
Portland State University

1989

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AN ABSTRACT OF THE THESIS OF Pamela Kay Thinesen for the Master of Science in Biology presented April 14, 1989.

Title: Seasonal Trailing Behavior and Corticosterone Levels in Male Red-Sided Garter Snakes (*Thamnophis sirtalis parietalis*)

APPROVED BY THE MEMBERS OF THE THESIS COMMITTEE:

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Mechanisms of how red-sided garter snakes (*Thamnophis sirtalis parietalis*) travel up to 18 km from summer feeding sites to hibernation dens are not understood. In this study, monthly and seasonal trailing behavior were investigated to determine whether red-sided garter snakes prefer to follow trails of snakes from the same den (den-mates) versus trails made by other conspecifics (non-den-mates). Snakes from five different hibernacula in Manitoba, Canada, were involved in the study. Eighteen were adults and 15 were subadults. Subadult red-sided garter snakes do not return
to hibernacula until their second year of life, so their trailing behavior was of interest in learning how they might first find hibernation sites.

Since hormonal factors may play a role in trailing behavior, and, since corticosterone (CS) has been associated with migration and homing in other vertebrates, plasma CS concentrations were also investigated monthly and seasonally.

A wooden Y-maze with slots for blocking the two arms was used in the study. Overall, the snakes trailed conspecifics in the spring but showed no preferences for trails of den-mates or non-den-mates. When adults' and subadults' behaviors were studied separately, no significant trailing tendencies were observed in the adults. Subadults preferred to follow den-mates in the fall and five weeks' post-emergence, corresponding with the time of migration to hibernacula and migration to summer feeding sites, respectively. Two-way analysis including age as a factor showed no age by month interaction; that is, age appeared to have no effect on CS levels during any particular month.
SEASONAL TRAILING BEHAVIOR
AND
CORTICOSTERONE LEVELS IN MALE
RED-SIDED GARTER SNAKES
(THAMNOPHIS SIRTALIS PARIETALIS)

by
PAMELA KAY THINESEN

A thesis submitted in partial fulfillment
of the requirements for the degree of

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in
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1989
TO THE OFFICE OF GRADUATE STUDIES:

The members of the Committee approve the thesis of

Pamela Kay Thinesen presented April 14, 1989.

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CHAPTER I

INTRODUCTION

In this study, I investigated trailing behavior of male red-sided garter snakes (*Thamnophis sirtalis parietalis*) from five different dens in the Interlake Region of Manitoba, Canada. I studied trailing both monthly and seasonally to compare this behavior during different periods. My goal was to determine whether snakes from a given population preferentially follow trails laid down by snakes from the same population and to determine differences in seasonal trailing patterns. Since snakes are generally born far from hibernation dens and do not travel to the dens until their second year of life (Gregory, 1971), subadult trailing behavior was also investigated. The goal, in this case, was to learn about possible mechanisms by which snakes return to the same dens each year and how subadults find the dens in their second year of life.

In addition, I investigated the corticosterone (CS) component of the snakes' hormonal condition to determine if there is a difference in hormonal state between snakes which show trailing preferences and in those which do not. Since migratory activity is generally seasonal, determining if plasma levels of CS change seasonally is also of interest. Significant fluctuations may indicate a correlation with
fall migrations to hibernacula and/or spring dispersal to feeding areas.
CHAPTER II

REVIEW OF THE LITERATURE

TAXONOMY AND NATURAL HISTORY OF

THAMNOPHIS SIRTALIS PARIETALIS

Thamnophis is one of 270 genera of squamate reptiles in the Family Colubridae and the Subfamily Natricinae (Nussbaum et al, 1983). It is the most abundant and widely-distributed genus of North American snakes (Garstka, 1982; Gregory, 1971).

_T. sirtalis_ is one of 22 known species of Thamnophis; thirteen species are found in the United States and Canada. Twelve subspecies of _T. sirtalis_ are generally recognized in the United States and Canada (Nussbaum et al, 1983), although the taxonomic status of this species needs revision. _T. sirtalis_ is most commonly found in meadows and marshes near streams, rivers, and lakes, but it also occurs far from water in valleys and in coniferous forests. Elevations at which the species occurs vary from sea level to 2450 meters above sea level (Fitch, 1965; Nussbaum et al, 1983).

The subspecies _T. s. parietalis_ (red-sided garter snake) is common in the Plains areas of North America and also occurs throughout the southern half of Canada (Nussbaum
et al, 1983). Dens consist of rocky areas containing small openings and crevices which the snakes enter for protection from predators and from subzero temperatures. Red-sided garter snakes are also found in the Interlake Region of Manitoba, Canada, where they use limestone sinks in the bedrock as hibernacula for six or more months of the year (Aleksiuk and Stewart, 1971; Garstka, 1982; Gregory, 1971, 1974, 1977, 1982; Lynch, 1983).

Hibernation appears to result in a decrease in metabolism of heart tissue and in heart rate (Aleksiuk, 1976), but physiological changes occurring before and during reptilian hibernation are not completely understood (reviewed by Joy and Crews, 1984, 1987). Mechanisms of the hibernation cycle are at least partly endogenous, in that snakes beginning hibernation in early or late summer do not survive as well in hibernation as snakes beginning hibernation in the fall (Joy and Crews, 1987).

Possible reasons for reptilian hibernation include avoiding the stresses of lethal temperatures and lack of food during the winter months (Gregory, 1982). Aggregational hibernation may help individuals conserve moisture and body heat (Gehlbach et al, 1971). Red-sided garter snakes do not survive at temperatures much below 0°C (Gregory, 1982), and they can withstand 0°C for only a few hours (Whittier and Crews, unpublished observations). In the Interlake Region of Manitoba, surface temperatures
frequently reach -40°C in winter months (Gregory, 1977; Joy and Crews, 1987).

The timing of migration to the dens to begin hibernation and of emergence from the dens at the end of each period of dormancy depends on climate in the various geographic regions. In general, however, each population returns to the same den in the late fall of each year and emerges in early spring (Gregory, 1971, 1977; Lynch, 1983). Temperature appears to play an important role in the snakes' annual cycle (Gregory, 1971; Lynch, 1983; Whittier et al, 1987b). Adult red-sided garter snakes in Manitoba often gather in groups of thousands per hibernaculum and generally arrive at the den sites from late August to mid-October, enter the dens several weeks after arrival, and emerge in March, April, or May (Gregory, 1971; Gregory and Stewart, 1975). Males emerge first in large groups; females emerge singly over a period of two to three weeks (Garstka et al, 1982) or perhaps throughout the entire spring season (Gregory, 1974). Mating occurs as the females emerge; females migrate to summer feeding grounds almost immediately after mating, while males disperse to the feeding sites three to four weeks after emergence (Garstka et al, 1982; Gregory, 1977; Mason, 1987). Wood frogs (Rana sylvatica) are the snakes' main prey, and marshy areas serve as their summer habitat.
Females are viviparous and give birth around mid-August; they return to their respective dens soon after (Gregory, 1971; Lynch, 1983). Time of birth varies with time of emergence in the spring, time of ovulation, and climate, especially environmental temperature. Interestingly, gestation can be prolonged past the normal three months by low air temperatures (Lynch, 1983).

Communal denning may ensure breeding success in *T. s. parietalis*, a species which has such a short active period in a harsh environment (Aleksiuk and Gregory, 1974; Gregory, 1971, 1974). The Interlake Region of Manitoba has a cool temperate climate which might be more accurately described as subarctic (Aleksiuk and Stewart, 1971). With communal denning, time and energy need not be spent searching for mates. The fact that only sexually active adults den communally lends support to this idea.

Individual red-sided garter snakes return to the same den each year, but how they do so is not understood (Gregory, 1971, 1977). It is possible that there is pheromonal communication between snakes of the same population which guides them back to the same dens each year, especially since the snakes may disperse 18 or more kilometers from their respective dens each spring (Gregory, 1977; Gregory and Stewart, 1975).

Of further interest is the finding that young snakes do not hibernate in traditional dens until their second winter.
of life (Gregory, 1974, 1977; Lynch, 1983). Unfortunately, little of the natural history of first-year red-sided garter snakes is understood, as they are rarely found (Lynch, 1983), and where they spend their first winter is not known. It is possible that first-year snakes continue feeding longer than do the adults and that the first-year snakes remain in summer feeding areas over the winter, hibernating in underground areas which adults, being larger, are unable to use (Gregory, 1977).

There would be little advantage for a sexually immature snake to migrate the lengthy distance to a communal den (Lynch, 1983), since red-sided garter snakes do not breed until the second year of life (Gregory, 1977). In Michigan, young snakes have been found hibernating in crayfish dens, vole tunnels, and anthills; similar areas may be used in Manitoba (Lynch, 1983). Some evidence indicates that the first time a garter snake hibernates in a communal den, it does not necessarily use the one which its parents used. However, once a snake has used a den, studies suggest it returns to the same den each year (Gregory, 1977; Lynch, 1983).

**PHEROMONAL COMMUNICATION AND ITS RELATIONSHIP TO TRAILING**

Pheromones are chemicals secreted externally by organisms which function in communicating information to other organisms. This type of communication plays an
important role in the lives of many organisms. Snakes' chemical communication systems can serve as a model for reaching an understanding of vertebrate systems, as the snakes' behavioral repertoire and sensory systems are relatively simple, well-understood, and easier to study than are those of other vertebrates (Mason, 1987).

In insects, pheromones are composed of esters that elicit varying behaviors (Mason, 1987). Decenoic acid and alcohol derivatives are recognized as sex pheromones in queen honeybees, female gypsy moths, and female silkworm moths (Jacobson and Beroza, 1963). Esters also serve as pheromones in the American cockroach (Jacobson et al, 1963).

In snakes, research has been conducted on sex pheromone trailing (Ford and Low, 1984), species specificity of sex pheromone trails (Ford, 1982; Ford and O'Bleness, 1986), conspecific scent trailing (Brown and MacLean, 1983), the evolution and adaptive significance of pheromones (Graves et al, 1986; Duvall, 1986), and avoidance of conspecific trails (King et al, 1983). Most workers have investigated sex pheromones, but Graves and Duvall (1984) found that prairie rattlesnakes (Crotalus viridis) communicate danger by secreting an alarm pheromone from cloacal sacs. Snakes exposed to alarmed snakes' cloacal discharges experience elevated heart rates and other behaviors which accompany defensive behavior.
Chemicals suggested as possessing pheromonal properties include alcohols; carboxylic acids; hydrocarbons; steroids; and, in garter snakes, a lipoprotein, vitellogenin (Garstka, 1982). However, the chemical nature of pheromones in snakes, and in reptiles in general, remains elusive (Mason, 1987). Skin lipids apparently serve as semiochemicals (Mason et al, 1987), but, to date, no reptilian pheromone has been identified chemically. The earliest studies on pheromonal communication in Thamnophis sirtalis were conducted by Noble (1937), who observed that male T. sirtalis and T. butleri followed trails laid down by skin secretions of estrous females. In the 1970s, Devine (reviewed by Mason, 1987) found that the female sex attractiveness pheromone consists of wax esters; esters are compounds made up of an acid and an alcohol which are often odorous. In the 1980s, it was thought that these wax esters were nonpolar lipids made up of 26 to 36 carbons (Mason, 1987).

Other studies have suggested that the sex pheromone in T. s. parietalis is vitellogenin or a component of the vitellogenin molecule. Vitellogenin is a lipoprotein yolk precursor produced in the liver in response to estrogen and present in the circulation of sexually active female red-sided garter snakes (Crews, 1976; Garstka, 1982; Garstka and Crews, 1981; Kubie et al, 1978). Females and estrogen-treated males have a lipid-rich skin layer; lipids become
concentrated in hinge regions, the points of scale attachment, which contain very thin regions of epidermis (Mason, 1987). The pheromones may travel from dermal vascular areas to the skin and then be secreted through these thin epidermal areas during hyperventilation, a behavior which occurs in females during courtship (Garstka and Crews, 1981; Mason, 1987).

As to the source of the pheromones in snakes, researchers have considered cloacal sacs, the skin, and fecal matter (reviewed in Mason, 1987). Cloacal secretions of garter snakes appear to be less important than skin secretions (Ferguson, 1977). For example, male Thamnophis sirtalis and T. butleri do not follow trails made by estrous female cloacal secretions but, as stated previously, do follow trails formed by estrous female skin secretions (Noble, 1937). Further, estrous females whose skin is coated with petroleum jelly are not courted (Noble, 1937).

Rauch (1978) has hypothesized that in Thamnophis, only female skin is structured in such a manner so as to allow blood-borne pheromones to be secreted. However, Garstka (1982) showed that male and female skin structure in Thamnophis is similar in that both sexes possess dermal lipid storage areas, and estrogen treatment in males produces lipid staining patterns similar to those seen in females.
In addition, male *T. sirtalis sirtalis* do not court females wearing skins of freshly killed males but do court males wearing female skins, which indicates that the integument may contain important chemical cues and that these cues differ between the sexes (Gillingham and Dickinson, 1980). This also indicates that the skin plays a role in sex identification. Mason (1987) found that only males' skin lipids contain squalene, a lipid precursor to cholesterol, and hypothesized that this is the component which allows males to identify each other as males.

Like many other squamates, *Thamnophis* detects pheromones laid down on the substrate by other snakes (Ford and Low, 1984); the tongue touches the substrate and then the vomeronasal organ (VNO) ducts for analysis (Gillingham and Clark, 1981; Halpern and Kubie, 1983). Studies indicate that snakes can detect the sex of other snakes by analyzing pheromones that have been left on the substrate (Ford and Low, 1984). Sexual receptivity and species identification, especially in sympatric species, can be communicated via pheromones, as well (Ford, 1982; Ford and Schofield, 1984; Ford and O'Bleness, 1986). It seems reasonable that sympatric species have evolved distinguishable differences in pheromonal components, as it is advantageous to be able to recognize one's own species' trails for purposes of reproduction. Trails may also be used for non-reproductive
activities such as aggregation (Dundee and Miller, 1968; Gregory, 1975; Noble, 1937).

Conversely, it is important to recognize trails of other species to avoid wasting energy following the wrong trail (when looking for a mate, for example). Individual T. sirtalis sirtalis apparently can distinguish their own cues from those of conspecifics (Heller and Halpern, 1982a). Responses of T. radix to conspecific and interspecific soiled areas in test chambers indicate that this species prefers areas soiled by conspecifics over clean areas (Porter and Czaplicki, 1974). Being able to identify conspecific chemical cues may contribute to dispersal and aggregation during the annual cycle (Porter and Czaplicki, 1974).

CHEMOSENSATION AND ITS RELATIONSHIP TO TRAILING

Visual, olfactory, and vomeronasal functions are the major communicatory mechanisms in reptiles (Heller and Halpern, 1982b). The VNO, also known as Jacobson's organ, is a paired structure found in members of the Order Squamata (lizards and snakes) (Simon, 1983), as well as in many other vertebrates such as insectivores, rodents, anurans, and urodeles (Garstka, 1982). The importance of VNO chemoreception in feeding, orientation, homing, exploratory, and sexual behaviors, and in discrimination between conspecifics and heterospecifics, has been studied in several species of

The VNO is located below the nasal passages and connected with the mouth through anterior palatal ducts (Simon, 1983). Olfaction and VNO structures, innervations, and functions are separate. The VNO is functionally related to the tongue, which flicks outward to pick up environmental cues, is drawn into the mouth, and transfers cues to the VNO ducts (Parsons, 1967, 1970; Burghardt, 1970; Burghardt and Pruitt, 1975; Cooper and Garstka, 1987; Kubie, 1977). Tongue-flicking activates the accessory olfactory bulb (Meredith and Burghardt, 1978). Vomeronasal neurons extend to the hypothalamus, which regulates production and release of anterior pituitary hormones (Halpern, 1976; Scalia and Winans, 1975). Forked tongues may be more effective than unforked tongues in transferring molecules to the VNO, because each tip can be inserted into one of the paired ducts (Simon, 1983).

Conversely, it has been hypothesized that, rather than the bifurcated tongue, two sublingual folds called anterior processes function in transferring cues to the VNO (Gillingham and Clark, 1981). For example, in the monitor lizard (Varanus albigularis) the forked tongue does not contact the VNO (Oelofsen and Van Den Heever, 1979). The ventral surface of the tongue of rat snakes (Elaphe)
contacts the anterior processes each time the tongue is retracted, and these processes are in direct alignment with the VNO (Gillingham and Clark, 1981). When the tongue is withdrawn into the sublingual sheath as the mouth closes, the anterior processes are raised up, thus contacting the two openings of the VNO. Although it might be said that these processes push the tongue into the VNO, filming of tongue retraction and elevation of the processes after retraction into the sheath shows that the anterior processes are probably the organs of chemical transfer to the VNO (Gillingham and Clark, 1981). Further, removing these processes in Thamnophis results in an inability to detect the presence of food (Gillingham and Clark, 1981).

For many reptiles, the VNO may be the most vital communicatory sensory system. Studies in which one of the three sensory systems is blocked show that snakes with intact VNO's exhibit normal behavior, even when both vision and olfaction are blocked. Such snakes successfully court (Kubie et al, 1978), trail prey (Kubie and Halpern, 1979), and display normal aggregative behavior (Burghardt, 1980). Such snakes also show conspecific avoidance indicating intraspecific competition (King et al, 1983). When the VNO is dysfunctional, these behaviors are less frequent or absent.

Alternatively, olfactory and VNO systems have been proposed to work together. Early studies showed that, in
male *Thamnophis sirtalis* and *T. butleri*, when the nostrils or VNO is coated with petroleum jelly, or when the tongue is severed, the snakes no longer courted estrous females (Noble, 1937). Olfaction appears to function at a low level, allowing long-term discrimination. This olfactory function, in turn, may activate the VNO. The VNO appears to function more specifically and acutely in short-term discrimination (Simon, 1985; Cowles and Phelan, 1958; Duvall, 1980, 1981). Olfaction appears to be more important in picking up volatile substances, while the vomeronasal system detects non-volatile or water-borne substances (Kubie, 1977).

Regardless of which sensory component is more important, the significance of the VNO in this study lies not in whether it is more important than olfaction or vision but in its general function in trailing behavior. It is well-established that many species of snakes follow trails of conspecifics (Burghardt, 1970; Ford, 1981; Ford and Schofield, 1984; Heller and Halpern, 1981; Mason, 1987). Rattlesnakes follow conspecific trails to hibernacula and when seeking mates (Klauber, 1956). Adult prairie rattlesnakes (*Crotalus viridis viridis*) avoid conspecific chemical cues, and such chemical cues may be involved in dispersal from dens after emergence from hibernation (King et al, 1983). The blind snakes (*Typhlopidae* and *Leptotyphlopidae*), and three species of colubrids (*Virginia*
striatula, Tantilla gracilis, and Sonora episcopa) follow their own individual trails when given a choice between their own and trails of conspecifics of the same sex (Gehlbach et al, 1971). Similarly, individual eastern garter snakes (Thamnophis sirtalis) enter and emerge from the same openings, and travel to and return from feeding areas along the same routes, each day (Gillingham and Rowe, 1984).

That the VNO in red-sided garter snakes is used to guide them on conspecific scent trails has been shown in several studies (Heller and Halpern, 1981; Ford, 1982; Ford and Low, 1984; Ford and O'Bleness, 1986), and characteristic changes in tongue-flicking occur when relevant pheromones are detected. These changes can readily be observed and have been used as criteria in trailing experiments; they include drawing the head back and rapidly flicking the tongue a portion of its full length towards the substrate. Such behavior is called a trail contact response (TCR) (Brown and MacLean, 1983; Ford and O'Bleness, 1986). After detecting the pheromones and while actually following the trail, the tongue extrudes its full length into the air (Brown and MacLean, 1983). However, there may be variation in tongue-flicking behaviors during trailing, and a particular pattern should not be used to indicate trailing (Cooper and Vitt, 1986c). Further, individual variation in tongue-flicking patterns and rates occurred in Thamnophis
radix observed for ten minute periods during a ten-day study (Chiszar and Carter, 1975). In racers (Coluber constrictor), trailing behavior includes searching movements of the head from side-to-side, except when following the precise route of the trail. Tongue-flicking occurs frequently while trailing (Lillywhite, 1985).

Individual variation in tongue-flicking also occurs in Typhlops, and some individuals do not tongue-flick at all while following a trail but make trail contact with their nares (Gehlbach et al, 1971). These blind snakes also follow conspecific trails better than colubrids do (in regard to excitability and distance followed). Both blind snakes and colubrids follow trails of conspecifics of the opposite sex farther than trails of the same sex or their own individual trails (Gehlbach et al, 1971). They also follow their own individual trails farther than they do trails of the same sex. Therefore, trailing of conspecifics, regardless of sex, may be important in migration and in aggregation for hibernation.

Most studies have examined males trailing females, although males have been observed to follow other males in studies involving typhlopids, leptotyphlopids, and colubrids (Gehlbach et al, 1971; Lillywhite, 1985).

Some studies question whether male snakes can lay trails. For example, male checkered garter snakes (Thamnophis marcianus) do not follow other males (Ford and
O'Bleness, 1986). Males of this species follow only females' trails, suggesting that only females lay trails and produce pheromones (Ford and O'Bleness, 1986). Further, these findings suggest that, in this species, trailing behavior and pheromones are important mainly in reproductive behavior. However, further studies are required at different times of year to examine these ideas more fully.

Male garter snakes can detect directional components of a trail by pheromones females leave on pegs in an arena (Ford and Low, 1984). When the pegs are reversed, males travel in a direction opposite that of the original trail, and males cannot determine trail direction when the pegs are removed. Direction may, therefore, be determined by objects present on the natural substrate when snakes leave chemical cues on such objects (Mason, 1987). *T. s. parietalis* is known to show directional preferences; a mass migration to the south occurs in spring and to the north in late summer and fall (Gregory and Stewart, 1975).

In another study, male *T. sirtalis* were observed to follow trails of conspecific females best in April, May, and June when tested monthly from April to October (Ford, 1981). The males did not trail females in late July, but some trailing was seen in August, September, and October.

It is possible that cues other than chemosensory ones are used in homing, separately from or in conjunction with chemosensation. For example, displaced lizards (*Sceloporus*
jarrovi) can use a sun-compass mechanism to home rather than chemoreception (Ellis-Quinn, 1984). However, the lizards were displaced from their habitat and were not given the opportunity to lay down trails. These methodological problems obscure the conclusions that can be made from the study.

The preceding studies suggest trailing mechanisms whereby red-sided garter snakes could find their way back to dens in the fall and to feeding grounds in the spring. A necessary requirement of such a mechanism is that recognizable trails must endure on the substrate long enough to permit detection from one season to the next. Another unanswered question is how the first snake finds its way to a den or to feeding areas (Mason, 1987) if others follow a fresh trail each season.

HORMONAL EFFECTS ON BEHAVIOR

Pheromones are chemicals which have communicatory function between organisms, while hormones are chemicals produced within organisms which have internal physiological functions. Underlying hormonal mechanisms involving the steroidal glucocorticoid corticosterone (CS) are of interest in seasonal trailing studies. CS has been related to migratory behavior and activity levels in several vertebrates, including European green frogs (Leboulenger et al, 1979), sea turtles (Morris, 1982; Owens et al, 1986; Owens

Seasonal rhythms in plasma CS concentrations have been reported in frogs (Leboulenger et al, 1979), salmonids (Shapovalov and Taft, 1954), carp (Ilan and Yaron, 1976), cobras (Tam et al, 1972), and birds (Meier and MacGregor, 1972). American toads (Bufo americanus) exhibit seasonal and daily CS rhythms, and the control appears to be unrelated to photoperiod (Pancak and Taylor, 1983). That is, toads maintained on 12L:12D cycles show seasonal rhythms similar to those of animals maintained under natural photoperiods. These animals show CS peaks in the spring and fall; in the spring, they migrate to breeding grounds; in the fall, they travel to winter burrowing sites. Also, daily CS surges occur a short time before daily peaks in locomotor activity. Increased locomotor activity requires higher blood glucose levels which occurs with an increase in glucocorticoid release.

In carp (Cyprinus carpio), the highest levels of CS occur in September (Ilan and Yaron, 1976). In cobras (Naja naja), CS levels are highest during March and April (the mating season) and decrease in summer and autumn, with the lowest concentrations occurring at the onset of winter (Tam et al, 1972).
In female *T. s. parietalis*, plasma levels of CS are high on emergence from hibernation, decrease during breeding and the summer, and then increase in the fall (Whittier et al, 1987a). High plasma CS concentrations occur in male *T. s. parietalis* on emergence from hibernation (Camazine et al, 1980; Hawley and Aleksiuk, 1976; Krohmer et al, 1987).

Synthesis from cholesterol and release of CS by adrenal cells occur in response to adrenocorticotropic hormone (ACTH) stimulation. ACTH is synthesized by the pars distalis of the pituitary in vertebrates (Norris, 1985). It is released from the pars distalis in response to hypothalamic corticotropin-releasing hormone (CRH), which itself is produced in response to neural or blood-borne stimuli (Ganong, 1963). Novel and stressful stimuli result in CRH release, ACTH secretion, and, thus, in CS release (Mason, 1968). Stressors include changes in light-dark cycles, temperature (Norris, 1985), and encounters with potential predators. CS increases glucose synthesis from amino acids and fat, resulting in an increased availability of glucose for responding to stress (Campbell, 1987).

Although not studied extensively, it appears that ACTH regulation in reptiles is homologous to mammalian regulatory mechanisms (Norris, 1985). Mammalian ACTH consists of a specific sequence of 39 amino acids; amino acids four to ten appear to be important in various learned behaviors (De Wied et al, 1975; Witter et al, 1981; Gispen, 1982). ACTH has
been positively correlated with rates of learning in rats; e.g., rates of learning avoidance behaviors have been shown to increase as has length of time before habituation occurs (Bohus and De Wied, 1981). Other behavioral manifestations of ACTH in vertebrates (including humans) include effects on learning, memory, motivation, attention and retention (Bohus and De Wied, 1981; Gispen, 1982; Witter et al, 1981).

ACTH and its cogeners may also enhance motivation and attention and responsiveness to environmental stimuli (Gispen, 1982). This is of interest in that such effects may aid vertebrates in migratory behaviors. Also of interest is the finding of an association between a decrease of food intake and an increase in CS in birds and salmonids before important events such as oviposition (Zadworny et al, 1986) and migration (Meier and MacGregor, 1972; Shapovalov and Taft, 1954). In domesticated birds, a decrease in food availability or reduced environmental temperatures is associated with an increase in plasma CS levels (Siegel, 1980). Plasma CS increases along with a decrease in food consumption by turkey hens during the two weeks before oviposition (Zadworny et al, 1986). This increase in CS may function in maintaining the hens nutritionally and in providing energy for egg production.

Elevated plasma corticosteroid levels occur in coho salmon (Oncorhynchus kisutch) in late May, during smoltification when they are migrating to the ocean (Specker and
Schreck, 1982). The salmon studied were maintained under constant temperature, so temperature cannot be the only cue resulting in increased corticosteroid release. It is hypothesized that increased corticosteroid levels function in increasing blood glucose and adaptation to sea water (Specker and Schreck, 1982).

CS peaks occur in European green frogs (Rana esculenta) in May and June, during the breeding season, and in late March when they emerge from hibernation (Leboulenger et al, 1979). Factors other than ambient temperature fluctuations, alone, contribute to changes in CS levels (Leboulenger et al, 1978). Increased ACTH sensitivity in September and decreased sensitivity in May occurs in carp (Cyprinus carpio), although this may be associated with age differences (Ilan and Yaron, 1976). Thus, at least in some vertebrates, seasonal variations in interrenal ACTH sensitivity occur.

Maximum CS levels have been found in frogs immersed in water when water temperature was at the seasonal low, while much lower CS levels were found in August, when water temperature was at its highest (Leboulenger et al, 1979). However, the same authors have also reported an increase in CS with increases in ambient temperatures (Leboulenger et al, 1978; Delarue et al, 1979). Increased CS levels are also associated with important physiological events which require high locomotor activity. Elevated CS levels have
been observed in lizards (Sceloporus cyanogenys) in May, corresponding to a period of increased locomotor activity (Daughtery and Callard, 1972). In addition, spotted newts (Notophthalmus viridescens) experience daily increases in CS which correspond with the drive to return to water (Meier et al, 1971).

ACTH and ACTH-like peptides and corticosteroids have also been shown to be involved in imprinting in ducklings. Peripheral administration of ACTH 1-10 facilitates ducklings' approaches to and establishment of a relationship with a moving figure, whether that moving figure is the mother or some other living or non-living object (Martin, 1981). Further, CS levels rise 12 to 24 hours after Peking ducklings (Anas platyrhynchos) hatch (Weiss et al, 1977). The increase in CS occurs at the end of the imprinting period and may be a source for increased fear response to animals other than the object that was imprinted on. Further, CS injections inhibit approach responses (Martin, 1981). CS is correlated with avoidance of new animals in a duckling's environment.

However, Martin (1975) observed a rise in CS after hatching and a decrease near the end of the period of imprinting in Peking ducklings. This is in direct opposition to the findings of Weiss et al (1977). Also, Martin suggests that ACTH-like peptides suppress avoidance responses and may facilitate approach responses to moving
objects. Martin used hormonal injections and observed ducklings' responses to various moving objects in the laboratory. Weiss et al kept the hatchlings in boxes devoid of light until killing them, collecting blood, and determining hormone concentration by competitive protein binding. This difference in experimental methods may be one contributing factor as to why contrasting conclusions were made.

Positive correlations have also been found between ACTH levels and frenzied enmass nest emergence of hatchling sea turtles down beach fronts to the ocean (Owens and Morris, 1985). Loggerhead hatchlings (Caretta caretta) have high CS levels at hatching and lowered levels for the first five days post-hatching, a time when they remain in the nests and during which a period of olfactory imprinting may occur (Morris, 1982). A surge of CS occurs on the fifth day, which corresponds to the "frenzy period" when the hatchlings leave the nests to enter the ocean (Morris, 1982). Whether sea turtles imprint on natal beaches via olfaction and/or other senses or follow experienced adults to the beaches remains unknown (Owens et al, 1982), as does whether turtles possess a pheromonal communication system (reviewed in Owens et al, 1982). In soft-shelled turtles (Lissemys punctata punctata), age apparently has no effect on daily adrenal hormone levels (Mahapatra et al, 1987). The degree of fluctuation of CS is very similar in juveniles and adults.
CS stimulates catabolism of glycogen, fat, and amino acids which may provide for certain energy-demanding events in the vertebrate life cycle. The cessation of food intake and the concomitant increase in CS concentration is of interest in that garter snakes stop eating before they enter hibernation. It is possible that CS levels rise and provide energy for the long trip back to the dens. However, in cobras (Naja naja), CS levels are low before entering hibernation, which facilitates energy storage via fat accumulation in preparation for hibernation; and a rise in plasma CS levels occurs in the spring (Tam et al, 1972). On emergence from hibernation, red-sided garter snakes are near starvation and may have lost up to a third of their prehibernation body weight (Lynch, 1983). Much energy is required at this time for the breeding season and then for the trip to summer feeding sites. The pituitary-adrenal system may play a role in providing the ability to adapt to these sources of stress.

The literature reviewed here indicates that a relationship may exist between trailing behavior, pheromonal and/or chemosensory communication, and hormonal components. My study is an attempt to explore further such relationships in male red-sided garter snakes, especially in regard to conspecific trailing behavior and its relationship to plasma CS concentrations.
CHAPTER III

DESCRIPTION OF THE RESEARCH QUESTIONS

One research question is whether adult male Thamnophis sirtalis parietalis preferentially follow trails made by other adult male den-mates. This was investigated seasonally and monthly.

A second question is whether subadult male red-sided garter snakes show any tendencies to follow trails of conspecific males. This was also studied seasonally and monthly.

My third question is whether there are correlations between trailing behavior and CS levels in T. s. parietalis, seasonally and/or monthly. Because migration of red-sided garter snakes to and from dens is seasonal, changes in trailing behavior accompanying changes in season are of interest. By analyzing plasma sampled at different times of the year, any significant correlations between seasonal changes in trailing preferences and CS levels can be determined.
CHAPTER IV

MATERIALS AND METHODS

GENERAL METHODS

Animals

Male red-sided garter snakes collected from five different dens in the Interlake Region of Manitoba, Canada, (Fig. 1) were used in the experiments. The snakes were obtained in the Spring and Fall of 1987. On their arrival at Portland State University, the snakes were marked by scute clipping, measured to the nearest centimeter from snout-to-vent, and weighed to the nearest gram (Table I). Thirty-three snakes were involved in the trailing study; of these, 15 were subadults, and 18 were adults. Subadults are under two years of age and are generally less than 45 cm in snout-to-vent length (Fitch, 1965).

Snakes added to the study in the Spring of 1988, due to deaths of some original animals following the Fall of 1987 study, were not bled for analysis of plasma CS. Data for two snakes which died soon after testing in the Fall of 1987 were excluded, as their CS levels were abnormally high. These snakes were lethargic and obviously in ill health; they showed minimal reactions to stimuli during testing.
Figure 1. Map of portion of Manitoba, Canada, showing locations of dens from which snakes were collected for trailing study. Latitude and longitude of Narcisse is 50° N, 97° W, respectively.
TABLE I

SUMMER, 1987, AND SPRING, 1988, MEANS, STANDARD DEVIATIONS (SD), RANGES (R), SNOUT-TO-VENT LENGTHS (SVL), BODY WEIGHTS (BW), AND LOCATIONS OF DENS FROM WHICH SNAKES WERE COLLECTED IN MANITOBA, CANADA

<table>
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<tr>
<th>Season</th>
<th>Den</th>
<th>N</th>
<th>Mean SVL (cm)</th>
<th>SD SVL</th>
<th>Mean BW (g)</th>
<th>SD BW</th>
<th>R</th>
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<td>4</td>
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<td>2.5</td>
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<td>5.4</td>
<td>12</td>
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<tr>
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<td>11.9</td>
<td>28</td>
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<tr>
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<td>Narcisse</td>
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<td>1.7</td>
<td>32.2</td>
<td>2.0</td>
<td>4</td>
</tr>
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<td>2.7</td>
<td>36.7</td>
<td>4.6</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>19</td>
<td>45.2</td>
<td>4.2</td>
<td>33.0</td>
<td>7.6</td>
<td>28</td>
</tr>
<tr>
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<td>4.5</td>
<td>27.6</td>
<td>6.8</td>
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</tbody>
</table>

Animal Maintenance

The animals were housed in glass aquaria in a laboratory at Portland State University under natural light conditions and ambient temperature (ranging from 22° to 30° C). Each den group was kept in a separate aquarium. They were put into artificial hibernation in a cold room maintained at 4°C in October, 1987, and removed from hibernation in February, 1988. When not in hibernation, they were kept on a substrate of wood chips in glass aquaria with overhanging Vita-Lights. Twice weekly, the snakes ate
fresh chopped fish supplemented with vitamins and minerals. Water was provided ad libitum.

SPECIFIC METHODS

Behavioral Testing

Control Tests. The control tests were conducted to determine whether snakes showed preferences for the left or right arm of a Y-maze (Fig. 2).

Fresh paper was placed in the bottom of the maze shaft and arms for each individual test. At the end of each arm of the maze was a petri dish containing ground fish, and the dishes were covered with a screen. A snake was placed in the shaft with his head oriented away from the arms towards the closed end of the shaft. He was given up to three minutes to turn toward the arms and to travel to and down one of the arms. This was done twice per snake per testing session in July, August, and September of 1987; and in February, March, and April of 1988. Observations were made by quietly leaning slightly over the maze wall in such a way that the animals could not see the observer.

To ensure that trailing was occurring in the Y-maze, males were tested against females as trail establishers; one arm was blocked, and a female was placed in the maze and allowed to make a trail down the open arm. The blocked arm was then opened, and a male was introduced to the maze and allowed to choose to travel down one of the arms. The paper
Figure 2. Y-maze used in trailing study. Its design is adapted from Heller and Halpern, 1981; Ford, 1982; Brown and MacLean, 1983; Ford and Low, 1984; Ford and O'Bleness, 1986. Major features include a wooden shaft; arm length, width, and height of 60 cm; and slots for blocking the arms with 60 cm high wooden inserts.
was changed, and the above was repeated with the opposite arm blocked and with each of the males.

**Trailing Tests.** The objective of the first experiment was to determine if male *Thamnophis sirtalis parietalis* preferentially follow a trail made by a member of the same population.

A snake was randomly chosen to be the trail establisher (TE). The TE was put into the shaft of the maze; one of the arms was left open and the other blocked with a wooden insert. The snake was given up to three minutes to move down the open arm towards the food, presumably leaving a trail (Fig. 3a).

The TE and wooden insert were then removed from the maze, and another snake was randomly chosen to be the trail follower (TF). He was released into the shaft of the maze and allowed to travel down the shaft towards the open arms (Fig. 3b). When his body, from head to vent, entered one of the arms, he was removed from the maze. Tongue-flicking responses were also observed to confirm that trailing was occurring.

If the snake followed the TE, he was given a score of +1; if he went down the opposite arm, he received a -1; and if he did not go down either arm within three minutes, he was given a zero. This was repeated twice per TE for each snake during each testing period. The arm that was blocked was alternated with each trial. One snake from each
population played the role of TE, and all the others were tested against each TE.

Figure 3. How trailing behavior was studied. (a) A snake (trail establisher) was placed at area A and allowed to travel to the food at the end of the open arm, presumably establishing a trail by leaving pheromonal cues on the substrate. The opposite arm was blocked with a wood insert (I). The snake was removed from the maze after establishing a trail. (b) Another snake (trail follower) was placed at area A. Both arms were open, and food was present at both ends. The snake was given up to three minutes to choose a trail.
The experiments were repeated once each month between July and September, 1987, and February and April, 1988. Monthly and seasonal trailing tendencies were determined. Results for adults, subadults, and both groups combined were examined. Three hundred seventy eight tests were conducted in the fall study and 281 tests were done in the spring study.

**Blood Sampling.** Caudal blood samples were collected from each snake into heparinized tubes after each month's experiments were completed. The blood was centrifuged to separate plasma from erythrocytes, and the plasma was stored at -20°C until July, 1988, when it was radioimmunoassayed for CS levels.

**Radioimmunoassay for CS.** Plasma CS concentrations were determined by radioimmunoassay (Whittier et al, 1987a) at the Oregon Regional Primate Center, Beaverton, Oregon. Accuracy was 98.4% with a sensitivity of 5 pg/tube. Comparisons were made between those snakes which showed a tendency to follow members of their own populations and those which showed no preferences. CS levels were also compared at different times of the year to determine whether there were significant differences.

**Statistical Methods.** Two-by-two contingency tables were set up, and one-tailed chi-square, Fisher exact, and sign tests (Siegel and Castellan, 1988) were used to determine whether there was a significant preference to
follow den-mates versus non-den-mates or to trail, in general.

Hormonal data were analyzed by one-way and two-way analysis of variance using log-transformed data and by Kruskal-Wallis nonparametric tests (Dixon, 1983; Sokal and Rohlf, 1981). A probability of \( \leq 0.05 \) was accepted as significant.
CHAPTER V

RESULTS

CONTROLS

With two exceptions, the snakes showed no significant seasonal preference for the right or left arm of the maze when no trail was present (fall, sign test, \( p=0.16, n=124 \); spring, \( p=0.25, n=114 \); Figs. 4A and 5A). One adult snake preferred the left arm (binomial test, \( p=0.02, n=9 \)), and another adult preferred the right arm (\( p=0.03, n=5 \)). These preferences were considered when the experimental data were analyzed; whenever these snakes followed a trail made in their preferred direction, the data were excluded from the results.

On a monthly basis, four adults showed a significant preference for the right arm in April (binomial test, \( p=0.01, n=30 \); Fig. 5C). This was considered when the experimental data were analyzed; the data were excluded in the same manner as for the individual preferences discussed above.

When a female garter snake was used as TE to determine whether trailing was occurring in the maze, 70% of males exhibiting significant courtship behavior (sign test,
Figure 4. Control tests, seasonal. Percentages of male Thamnophis sirtalis parietalis that chose right or left arm of Y-maze when no trail was present. A=All snakes; B=Subadults; C=Adults.
Figure 5. Control tests, monthly. Percentages of male *Thamnophis sirtalis parietalis* that chose right or left arm of Y-maze when no trail was present. *=Significant; A=All snakes; B=Subadults; C=Adults.
p=0.002, n=10) also tended to follow the female (Fig. 6), although this trailing behavior was not statistically significant (p=0.17). In another test, when most males were not exhibiting courtship behavior (sign test, p=0.003, n=38), there was no tendency to follow (p=0.44). The few males that did court (5 of 19 snakes, or 10 of 38 total trials, = 26.3%) showed no tendency to follow. In other words, 50% of those which courted also followed. In this latter test, the males had been out of hibernation for several weeks, and males normally cease courtship behavior two to four weeks after emergence from hibernation. They had also begun eating, an event that occurs after the breeding season.

![Bar chart](image)

**Figure 6.** Control tests. Trailing behavior of male *Thamnophis sirtalis parietalis* when female conspecifics were used as trail establishers. All recently emerged males were sexually active.

When results of the above tests are combined, 60.0 percent of the males that courted females also followed
the females in the maze. This relatively high percentage of trailers, plus my observations that they paused when a decision had to be made regarding which arm to travel down and tongue-flicked before and during arm choice decisions, indicate that trailing occurred.

TRAILING BEHAVIOR

The snakes showed no significant preference to follow den-mates or non-den-mates in the fall (sign tests, when the TE was from the same den, p=0.24, n=50; when the TE was from a different den, p=0.17, n=328; Fig. 7A), but they did trail both in the spring (p=0.04, n=275). They tended to prefer to follow den-mates in the spring (p=0.07, n=82).

On a monthly basis, the percentage that followed both den-mates and non-den-mates was greater than 50 percent for all months (Fig. 8A). With the exception of September and February, more than 60 percent followed den-mates, although this was not statistically significant (sign tests, p ranged from 0.08 to 0.30 and n from 15 to 24).
Figure 7. Trailing behavior, seasonal. Expressed as percentage of male Thamnophis sirtalis parietalis that follow when trail is established by an animal from same, different, and either den. *=Significant; A=All snakes; B=Subadults; C=Adults.
Figure 8. Trailing behavior, monthly. Expressed as percentage of male *Thamnophis sirtalis parietalis* that follow when trail is established by an animal from same, different, or both dens. * = Significant; A = All snakes; B = Subadults; C = Adults. N's less than ten are indicated.
Of interest is a decreasing tendency to follow both den-mates and non-den-mates as fall progressed. That is, 55.1% (sign test, p=0.12, n=147) followed in July; 53.5% (p=0.25, n=114) followed in August; and 50.4% (p=0.50, n=117) followed in September. An increasing tendency occurred as spring progressed. That is, 53.8% (p=0.23, n=119) followed in February; 57.3% (p=0.11, n=82) followed in March; and, in April, 56.8% (p=0.15, n=74) followed. In March, there was a higher tendency to follow den-mates than non-den-mates (66.7%, p=0.08, n=24; and 53.4%, p=0.35, n=58, respectively).

In contrast to adults, subadults preferred to follow den-mates in the fall (sign test, p=0.02, n=15; Fig. 7B). No such preference to follow was seen in the spring, although a tendency to follow both den and non-den-mates was observed (p=0.08, n=131).

On a monthly basis, the subadult snakes tended to follow den-mates in August (sign test, p=0.07, n=4; Fig. 8B) and in September (p=0.24, n=2), although the number of trials was low. In March, subadults preferred to follow den-mates, and this was statistically significant (p=0.02, n=12).

Adult male Thamnophis sirtalis parietalis showed no significant tendencies to trail (sign test, fall, p=0.37, n=252; Fig. 7C). In the spring, adult males tended to
follow den-mates (p=0.07, n=36). Also, adults tended to follow den-mates in April (p=0.09, n=9; Fig. 8C).

CORTICOSTERONE LEVELS

No correlation between trailing activity and plasma CS levels was found when log-transformed data were analyzed by one-way analysis of variance and nonparametric Kruskal-Wallis tests. Two-way analysis of variance was conducted including age as a factor, and this showed no age by month interaction; that is, age appeared to have no effect on CS levels during any particular month.

Plasma CS concentrations were highest in February (Fig. 9), shortly after emergence. CS levels were higher in the fall than in the spring. However, no correlation was seen between plasma CS concentrations and trailing behavior during any month.

Figure 9. Mean monthly plasma corticosterone levels. Bars labeled with the same letters are not significantly different from one another. Standard deviations are as follows: February, 39.6; March, 16.3; April, 13.8; July, 44.3; August, 27.2; September, 26.0.
CHAPTER VI

DISCUSSION

TRAILING BEHAVIOR

These studies support the hypothesis that male *T. sirtalis parietalis* lay pheromonal trails on the substrate. First, sexually active males tended to follow females in the Y-maze in the control tests. Second, males followed trails in the experimental tests. These results are further supported by behavioral observations; snakes that trailed followed the exact path made by the TE. Furthermore, trail followers exhibited characteristic tongue-flicking typical of trail detection.

Trailing in this species may vary with season. When data for subadults and adults are combined, snakes tended to follow trails of den-mates and non-den-mates in the spring. In nature, spring is the time of dispersal of these animals from dens to feeding grounds.

Subadult males significantly preferred den-mates' trails in the fall and in March. This suggests that male subadult red-sided garter snakes are able to recognize den-mates' trails and to distinguish den-mates from non-den-mates. Subadults must locate a den for the first time in the fall of their second year of life. Thus, trailing
behavior of these individuals may differ from that of experienced adults. That subadults trailed den-mates in the fall of the year may indicate that naive subadults rely more on chemical cues when homing than do adults. Thus, subadults can distinguish trails of snakes from the den in which they probably first hibernated. An alternative interpretation of these results is also possible; perhaps trail-following occurs at different times for adults and subadults, and this study was conducted during the time that subadults follow trails.

On a monthly basis, subadults followed trails of den-mates in March. This is when red-sided garter snakes used in this study would generally leave dens to migrate to feeding grounds. (The March tests were done five weeks after removal from the cold room, coinciding with the length of the breeding period and the time of dispersal.) Further evidence of this change is indicated by the onset of feeding behavior in the laboratory animals in March.

The adult male red-sided garter snakes did not trail conspecifics in the fall, which suggests that other cues may be important in homing, or that testing periods did not overlap with the times in which adults follow trails. Further evaluation of subadult and adult trailing behavior at more frequent intervals in the fall may elucidate these patterns.
CORTICOSTERONE LEVELS

There appears to be a seasonal pattern in plasma CS levels in male T. s. parietalis. High plasma CS concentrations occurred in February and in the fall, corresponding with periods requiring energy. (On emergence, the snakes engage in courtship and mating; in the fall, they migrate up to 11 miles to hibernacula.) High plasma CS levels also occur in cobras on emergence from hibernation (Tam et al, 1972) and in birds and salmon before and during migration to winter feeding sites and to the ocean, respectively (Meier and MacGregor, 1972; Shapovalov and Taft, 1954; Specker and Schreck, 1982). Similar changes in CS concentrations have been observed in toads (Pancak and Taylor, 1983), frogs (Leboulenger et al, 1979), lizards (Daughtery and Callard, 1972), newts (Meier et al, 1971) and in T. s. parietalis (Whittier et al, 1987a). High plasma CS levels occurring on emergence and the decreases seen in March and April are consistent with CS levels observed in other studies involving T. s. parietalis (Camazine et al, 1980; Hawley and Aleksiuk, 1976; Krohmer et al, 1987).

Interestingly, cobras experience a rise in plasma cholesterol on emergence and in the fall (Lance, 1975), corresponding to the increased plasma CS concentrations seen in T. s. parietalis during the same periods. Increases in cholesterol have also been observed in sockeye salmon.
(Oncorhynchus nerka) and in European eels (Anguilla anguilla) with onset of spawning; in amphibia (Xenopus laevis) before ovulation; and in white-crowned sparrows (Zonotrichia leucophrys gambelii) during the breeding season (reviewed in Lance, 1975). This is of interest in that CS is synthesized from cholesterol, and patterns in plasma CS concentrations observed in other studies correspond to these cholesterol concentrations.

Also of interest is the observation that the lower plasma CS concentrations seen in March and April correspond with low androgen levels observed during the breeding season (Camazine et al, 1980; Hawley and Aleksiuk, 1976; Krohmer et al, 1987). However, the meaning of this is not clear.
CHAPTER VII

CONCLUSIONS

TRAILING BEHAVIOR

The tendency of male *T. s. parietalis* to follow female conspecifics agrees with findings in several studies (Ford, 1981, 1982; Ford and Low, 1984; Noble, 1937). Few studies have been conducted to determine whether male colubrids follow male conspecifics (Ford and O'Bleness, 1986; Gehlbach et al, 1971), and none have been conducted on male *T. s. parietalis*. Only the subadult male red-sided garter snakes showed a significant tendency to trail male den-mates in the fall. These snakes were collected in the fall near den-sites; since they were captured in the fall, it is assumed that they had recently traveled to these dens for the first time in their lives. The results of this study suggest that there is some form of discriminatory ability in subadult red-sided garter snakes.

The finding that adults did not trail may indicate a dependence on other cues in homing. Alternatively, since the snakes were kept in a laboratory at room temperature in the fall, and migration to dens may be triggered by low night temperatures (Whittier et al, 1987b), it is possible that no stimulus to return to hibernacula occurred.
Subadults tended to follow den-mates in March. Since, at this time, they had been out of hibernation for five weeks, this coincides with the drive, in nature, to travel to summer feeding sites.

This study suggests that male T. s. parieta1is lay trails and that trailing varies with time of year, at least in the case of subadults. An interesting new observation from this work is the preference of subadults for den-mates' trails in the fall, when migration to hibernacula occurs; and five weeks after emergence, when dispersal to feeding sites would occur in nature.

CORTICOSTERONE LEVELS

Although no correlation was found between plasma CS levels and trailing preferences in this study, the monthly fluctuations in CS levels observed are consistent with findings in other studies involving this species (Camazine et al, 1980; Hawley and Aleksiuk, 1976; Krohmer et al, 1987; Whittier et al, 1987a). A correlation cannot be ruled out based on this study, alone; perhaps future investigations will show some relationship between plasma CS levels and trailing behavior.

The fluctuations in plasma CS concentrations also correspond to activity levels, as have been observed in other studies. High CS levels in the fall and on emergence may reflect a need for energy for homing and breeding.
LITERATURE CITED


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