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An Assessment of Environmental Enrichment on Morphology and Behavior of Yearling Rat Snakes (Elaphe obsoleta)

Lynn M. Almli
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To the Graduate Council:

I am submitting herewith a thesis written by Lynn M. Almli entitled "An Assessment of Environmental Enrichment on Morphology and Behavior of Yearling Rat Snakes (Elaphe obsoleta)." I have examined the final electronic 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 Ecology and Evolutionary Biology.

Gordon M. Burghardt, Major Professor

We have read this thesis and recommend its acceptance:

Jim Hall, Neil Greenberg

Accepted for the Council:
Dixie L. Thompson

Vice Provost and Dean of the Graduate School

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

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Anne Mayhew
Vice Provost and Dean of Graduate Studies

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An Assessment of Environmental Enrichment on Morphology and Behavior of Yearling Rat Snakes (*Elaphe obsoleta*)

A Thesis Presented for the
Master of Science Degree
The University of Tennessee, Knoxville

Lynn M. Almli
May 2004

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Numerous undergraduate researchers made this thesis project possible – too many to name individually. However, two students were indispensable in the last year of my study: Jon Paul Plumlee was always willing to assist in any and every aspect of this project, and Eric Ferrante was also at my beck and call for animal maintenance and any last minute frantic request. I am thankful to Shannon Stanley who assisted in helping me maintain my animals and whose job in the Reptile Ethology Laboratory allowed me to focus on my project.

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Abstract

Behavioral consequences of differential experience relating to studies of environmental enrichment have been documented primarily in mammals and birds. Similar data on experience-dependent behavioral plasticity are lacking in other vertebrates, especially non-avian reptiles. This project examined whether environmentally induced change occurs in snakes. Specifically, I housed rat snakes, *Elaphe obsoleta,* in enriched and standard environments to determine if differential experience can alter body morphology and improve behavioral abilities. Rat snakes are a particularly good model for this type of experiment because they are typically solitary and live in a complex three-dimensional habitat.

After being housed in different conditions for eight months, 16 *E. obsoleta* were measured and behaviorally tested in a feeding task, exploratory task, and a learning task. The results of this study demonstrate that housing condition, including feeding regime, can alter the morphology and behavior of captive snakes. In particular, snakes raised in enriched environments were larger (in mass and snout-vent length) and had increased growth rates as compared to controls. In a feeding task with live prey, snakes raised in enriched environments had shorter consumption times, suggesting increased foraging efficiency. In an exploratory task, snakes raised in enriched environments had higher initial tongue flick scores per trial and habituated more quickly to repeated exposures to the open field as compared to controls. Additionally, snakes raised in enriched environments maintained shorter latencies to the goal hole in a learning task, demonstrating superior learning ability as compared to control snakes, though neither group improved over the few trials conducted.

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Chapter 1: INTRODUCTION AND PURPOSE

The behavior of all organisms is dependent, in varying ways, on environmental factors and experience. These environmental stimuli or life events may be critical for development of species typical behaviors and thus for growth, maintenance, and reproduction. Determining the ways in which environmental factors can influence behavior is a daunting but necessary task. Laboratory and zoo studies have begun to make progress in this field.

The knowledge that enriched experiences may be necessary for the growth of species-specific brain characteristics and for obtaining full behavioral potential (Rosenzweig & Bennett, 1996) is not a new concept in science, although it has received much attention in the past few decades. Although the term "environmental enrichment" began after Hal Markowitz's pioneering work in the 1970s (he used operant conditioning techniques to improve the lives of captive animals; Markowitz, 1982), the groundwork for this concept was laid much earlier. For example, early ethologists, such as Lorenz (1937, 1950), recognized that animals have an innate need to perform natural behaviors. This led to the belief that preventing animals from performing these appetitive behaviors may be frustrating or stressful. In fact, abnormal behaviors called stereotypies (repetitive behaviors with no obvious goal or function) were prevalent in captive environments such as zoos (Hediger, 1964). Recent work in zoo biology has shown that environmental enrichment can ameliorate abnormal behaviors caused by impoverished conditions and can even prevent stereotypic behavior from occurring (see reviews by Shepherdson et al., 1998; Mellen & MacPhee, 2001).

Psychology laboratories have provided further insight into the field of environmental enrichment by conducting rigorous experiments in standard laboratory paradigms. This type of research began with Hebb (1949), who conducted one of the first experiments on the consequences of enriched rearing on the behavior of the rat by raising the rats in his home. In the 1960s, researchers from Berkeley (see Renner & Rosenzweig, 1987) began using a complex environment housing paradigm developed by Hebb (1949) as a tool for investigating environmentally induced change. In general,

animals exposed to enriched environments tend to have superior information-gathering abilities evidenced by their increased problem solving ability, increased exploratory behavior, and decreased emotionality (e.g., Zimmermann et al., 2001). Scientists have focused on two behavioral tasks to illuminate the benefits of environmental enrichment: the open-field task (for exploration) and the Morris water maze (for learning ability).

In general, enrichment studies have focused on the effects of the environment exclusively in mammalian and avian systems. This leaves open the question of whether the results are restricted to only these species. By studying several species' reactions to a standard experimental manipulation, it becomes possible to separate effects common across species from those that are unique to a particular species. For example, previous studies in rodents have attempted to dissociate the environmental factors (physical versus social stimulation) which contribute to the observed behavior changes from enriched experiences (van Praag et al., 2000). Because rodents are social animals, typical studies include group housing as part of their enriched treatment. Social stimulation becomes a confounding variable when determining the factors resulting in these changes. In other words, rodents may not be an appropriate model species for this type of study. I conducted an enrichment study in snakes because snakes are precocial and typically do not live in social groups (Brattstrom, 1974). Any behavioral or anatomical changes observed in this study of enrichment will be due solely to non-social factors in the environment.

Another rationale for conducting enrichment studies in non-avian reptiles is that the species have frequently been overlooked in assessments of psychological well-being (but see Burghardt et al., 1996; Marmie et al., 1990; Chiszar et al, 1993). For example, reptiles exhibit tachycardia when handled, which is similar to the effects observed with emotional fever in mammals and birds (Cabanac & Cabanac, 2000). Because environmental enrichment tends to decrease emotionality in rodents (Renner & Rosenzweig, 1987), it would be beneficial to investigate this phenomenon in reptiles. In addition, Burghardt et al. (1996) reported a Nile soft-shelled turtle maintained in captivity reduced self-mutilation behaviors after "play" objects were introduced into the enclosure. David Chiszar and collaborators have conducted a series of experiments on the

behavioral competence of captive rattlesnakes. Marmie et al. (1990) demonstrated that rearing snakes in small cages did not have debilitating consequences on locomotor behavior or chemosensation. However, in a predatory context, the captive reared rattlesnakes were impaired in strike induced chemosensory searching (SICS) as compared to wild caught animals. Furthermore, Chiszar et al. (1999) rescued six underweight *Crotalus viridis* from substandard housing conditions and discovered that they had depressed SICS as compared to wild caught snakes. After two years in appropriate housing conditions, normal foraging behavior in these snakes had been restored.

To address whether environmentally induced change occurs in snakes, I housed yearling rat snakes in enriched and standard captive environments to investigate if differential experience can alter body morphology and improve behavioral processes. In order to optimize comparisons between taxa and speculate on the phylogenetic distribution of environmentally induced plasticity in behavior, the experimental design was modeled after studies involving rodent subjects (the most widely studied animal in enrichment research) as well as previous work on the role of experience in snake behavior. Thus, the following behavioral designs were used: a feeding task with live and dead prey, an exploratory task in an open field (Almli, unpublished study; Chiszar et al., 1976), and a learning task based on both foraging and escape behaviors (Holtzman et al., 1999). Furthermore, the experiments were also designed to manipulate (or take advantage of) natural behaviors in an attempt to emulate Greenberg's ethologically informed design (EID; 1994). EID incorporates Tinbergen's four key factors (1963) when investigating behavior: causation, function, ontogeny, and evolution.

Rat snakes (family Colubridae; genus *Elaphe*) are a particularly good model to investigate environmental influences on the brain and behavior because their activity is dependent on spatial aspects of their environment (Mullin, 1998). For example, rat snakes are predators of nesting birds and small mammals in wooded landscapes and thus are active in both trees and under substrates (Weatherhead & Hoysak, 1989; Fitch, 1963). Furthermore, the fact that they constrict their prey adds to the diversity of their modes of interaction with the environment as well.

Morphological plasticity

Non-avian reptiles are good models for studies that seek to identify the relative roles of genetics and environment on morphology because they show a high degree of ontogenetic plasticity (Bonnet et al., 2001). Many animal species show extensive morphometric shape variation both within and among populations. Although this phenomenon has attracted considerable scientific attention, most studies have aimed at identifying its adaptive significance, and it is still unclear to what extent morphometric shape variation is environmentally induced. However, many studies have shown that different feeding regimes have induced variation in body size and head shape in a wide range of animals.

In snakes, changes in body size, length, and head shape are often attributable to food quantity (Forsman, 1996), diet (Krause et al., in press), and prey size (Forsman, 1991; Queral-Regil & King, 1998). For example, Forsman (1991) demonstrated variation in head length among mainland and island populations of European adders (*Vipera berus);* adders inhabiting islands with large voles had longer heads than those living on islands with smaller voles. Additionally, water snakes (*Nerodia sipedon*) feeding on large fish had greater body and head sizes than snakes that ate an equal number of smaller fish (Queral-Regil & King, 1998). In a laboratory study, Forsman (1996) reported significantly greater body sizes of snakes fed twice weekly compared to snakes fed once weekly on same species of prey; however, no size-independent variations in head dimensions were found. Bonnet et al. (2001) demonstrated that food availability during juvenile life affects not only growth rate but also the allometric relationships among body length, head length, and head width.

Head and body morphology was measured in this study to see if in fact housing environment induces morphological plasticity. This change in bone morphology may be a consequence of differential mechanical strain placed on jaw muscles during feeding (Lanyon & Rubin, 1985). Studies performed in fish have demonstrated that the kinematics of feeding induced by prey type may alter bone morphology. For example, the body and fin sizes of Trinidadian guppies (*Poecilia reticulata*) can be altered experimentally by manipulating the body orientation that fish must adopt in order to

forage (Robinson & Wilson, 1995). Additionally, Wimberger (1992) fed different food items (brine shrimp, flake food, and chironomid larvae) to neotropical cichlids. These food items required differing amounts of manipulation which could have caused the observed changes in jaw and skull measures. In this study, although the same size prey was fed to both treatment groups, the prey differed in activity levels (see below), which may have an effect on bone morphology of the enriched snakes.

Behavioral plasticity

Feeding study:

Most snakes, being limbless, do not manipulate objects (a common stimulator in enrichment studies) except with the head during feeding. Constrictors, such as the species studied here, also manipulate prey with their bodies by positioning coils in order to restrain, kill, shape, and maneuver prey. Many researchers have suggested that prey size and type have an effect on prey handling behavior in snakes (Greene, 1977, Loop & Bailey, 1972; Mori, 1996; de Quieroz, 1984). Loop and Bailey (1972) demonstrated that the size of the prey determined the probability of head first ingestion and prey capture technique, however, this finding could have also been due to ontogenetic differences in prey type. In fact, de Queiroz (1984) showed that helplessness of prey, independent of its size, has an effect on prey handling. Furthermore, he suggested that *Pituophis melanoleucus* were able to change their prey handling behavior to match the activity levels of their prey.

Several studies have investigated the effects of deprivation of live prey in snakes and obtained mixed results. When *Elaphe obsoleta* were deprived of live prey for almost one year, Milostan (1989) found no detrimental effects on prey handling ability caused by this lack of experience (e.g., deprived snakes demonstrated similar constriction patterns as normal snakes). Mori (1996) found a similar result when he raised *Elaphe quadrivirgata* on a diet of beef liver for over six months. As yearlings, these snakes showed differences in prey handling skills (they did not have a preference for head first ingestion, nor did they kill large mice before ingestion) but the differences did not result in shorter feeding latencies. Prey movement in addition to chemical cues may facilitate

prey detection and thus feeding efficiency (in *Thamnophis sirtalis,* Burghardt and Denny, 1983); it is surprising that there were not any observed deficits in feeding times in either Milostan or Mori's studies.

Nevertheless, even highly precocial species, such as rat snakes, may require feeding experience in order to forage efficiently, and feeding proficiency is crucial to survival in these animals (Greene, 1977, Burghardt & Krause, 1999; Krause & Burghardt, 2001). Mori's results (1996) in *Elaphe* further demonstrate that experience is necessary for development of prey handling skills in generalist snake predators; for example, after feeding experience *Elaphe quadrivirgata* (dietary generalist) were equal in rodent handling to *Elaphe climacophora* (rodent specialist). Improvements in foraging ability through ontogeny have been examined in many vertebrates (see examples in Burghardt & Krause, 1999) and typically involve differences in prey selection or increased efficiency in handling particular prey. During ontogeny, maturation in coordination and increased strength and size may all contribute to these improvements in foraging ability.

In this study, I investigated feeding experience and housing design on foraging efficiency. As typical captive environments do not provide opportunities to search for or manipulate prey, animals exposed to enriched experiences (e.g., live prey) may be more efficient feeders. Additionally, the potentially advanced musculature and coordination imposed by an activity in a stimulating environment may allow enriched snakes to be more adept at prey handling.

Exploration study:

Many researchers have used exploration studies for studying natural behavior and brain function because animals have an innate tendency to explore and may search for food even when it is readily available (Hughes, 1997). Appetitive behavior of this kind may provide information about the location and quality of future potential foraging sites in patchy environments (Shettleworth, 1998). In addition, animals will also explore familiar or novel environments, even when those environments contain no resources used by the animal during the period of exploration (Shettleworth, 1998). Investigative

exploration has also been shown to provide information important for predator avoidance strategies (Hughes, 1997).

Though controversial, the open field task has been employed by both biologists and psychologists to study exploration. An open field task consists of the measurement of behaviors elicited by placing the animal in a novel environment from which escape is prevented. Although initially designed to examine 'emotionality' in animals for which defecation served as a marker (see Walsh & Cummins, 1976), researchers gradually began to use the open field task to determine ways in which animals explore or recognize novel stimuli in the environment (see Walsh & Cummins, 1976). The first open field experiments designed to measure the tendency to explore a novel environment were performed with mammals, particularly rodents. Thus, the parameters developed to determine exploratory levels - ambulation, rearing behavior, freezing (immobility), and defecation - were rodentocentric (see the critique of open-field behavior in Suarez & Gallup, 1981).

Although Glickman and Sroges (1966) provided the preliminary framework for novelty testing in animals, their results led to many misconceptions about curiosity (a potential motive of exploratory behavior) in the "lower" vertebrates. They found reptiles in captivity to be "generally unresponsive" to objects that were placed in their cages in the zoo; the objects included lengths of chain, wooden dowels, and rubber tubing. Subsequent studies attempted in an effort to develop more appropriate diagnostic measures of exploration in non-avian reptiles, such as measuring tongue flick rates (e.g., Chiszar & Carter, 1975; Herzog & Burghardt, 1986). Tongue flicking in reptiles increases in novel habitats and thus may function in the acquisition of ecologically relevant chemical information (Greenberg, 1993). In snakes, tongue flick rates are a putative measure of level of interest in the environment, also correlated with locomotion, as in Burghardt & Pruitt (1975). For example, Chiszar et al. (1976) demonstrated that *Thamnophis sirtalis* have higher rates of tongue flicking when placed in a novel environment as opposed to just being handled and placed back in their home cage. Greenberg (1993) showed similar results in a lizard, *Anolis carolinensis.* Furthermore, satiated snakes habituated more rapidly than hungry snakes during exploration of an open

field thus exploratory behavior is related to similar factors that mediate foraging behavior (Chiszar et al., 1976). Burghardt et al. (1986) demonstrated that tongue flicking by iguanid species is suggestive of exploratory behavior and can even manifest in the field. Additionally, exploratory behavior is like most behavior in that individuals can differ greatly in their response to repeated exposures to the same environment (Chiszar & Carter, 1975).

In this project, an open field apparatus was used to determine if exploratory behaviors were altered by the presence or absence of environmental enrichment. The behavioral measurements used to determine exploratory levels were modeled after other open-field studies and included the following: number of tongue flicks, number of grid crosses, latency to escape-rear, duration of escape-rearing, and number of rearing bouts. A previous study of open field behavior in snakes revealed a behavior that I termed "escape-rearing" (Almli, unpublished study). Escape-rearing is a behavior in which the snake is moving in a vertical plane along the inside of the apparatus.

Learning study:

Experience with enriched environments may result in both latent learning and enhanced learning and cognitive abilities. The nature of learning and cognition in animals is an area of active research, although the view that learning is due to prior experience and involves changes in the nervous system is generally accepted (Shettleworth, 1998, Greenberg, 1993). To assess learning capacity, enrichment studies in rodents have relied on the Morris water maze to determine whether enriched animals are superior learners. The premise of the Morris water maze is that animals are motivated to learn cues go to a single goal (their motivation was to escape a "less rewarding" situation). These studies in rodents have demonstrated that animals raised in enriched environments perform better in the Morris water maze by learning to escape the water in a shorter time and by a more direct path (see Renner & Rosenzweig, 1987).

Choosing a particular technique to elucidate differences in learning ability in snakes exposed to differential housing treatments was difficult. It has long been thought that ectothermic reptiles have impoverished learning capacities (a view heavily criticized

in Burghardt, 1977), resulting in their being overlooked as animal models for certain behavioral tasks. The learning studies in reptiles reviewed by Burghardt (1977) primarily involved operant, associative, and maze learning. Learning studies in reptiles that have been most successful and reliable have used ecologically relevant cues (Brattstrom, 1978).

A recent trend in the learning literature involves spatial cognition (see review by Shettleworth, 1998) and with appropriate cues, reptiles reveal the ability to navigate to a goal with training (Day et al., 1999; Holtzman et al., 1999). To measure learning ability in snakes with differential housing experience, I adapted the apparatus and protocol developed by Holtzman et al. (1999) for spatial learning in red rat snakes – a task comparable to the Morris water maze. Holtzman's task was relatively devoid of ecologically relevant stimuli; thus, I added odor cues to the apparatus due to snakes' reliance on chemosensory information. Learning was determined by successful escape from the arena over numerous exposures to the apparatus.

Hypotheses

The following hypotheses were tested in this enrichment project:

Morphological plasticity:

1. Snakes raised in enriched environments will grow larger (e.g., increased mass and snout-vent length) as compared to controls.
2. These snakes will also have larger head dimensions (head width, head length, and jaw length) as compared to controls.

Behavioral plasticity:

1. Snakes raised in enriched environments will have increased foraging efficiency with live and dead prey as evidenced by a decreased consumption time as compared to controls.
2. Snake raised in enriched environments will exhibit increased exploratory behavior, but faster habituation, in an open field task (using rates of tongue

flicking and grid crossing) as compared to controls (in rodents, see Zimmermann et al., 2001).

3. Snakes raised in enriched environments will display improved learning ability by finding the goal hole in a shorter time than the controls in a Barnes maze (in rodents, see review by Renner & Rosenzweig, 1987).

Chapter 2: MATERIALS AND METHODS

Part 1 General methods

Subjects:

The subjects were 18 yearling rat snakes. Eight were captive born *Elaphe obsoleta quadrivittata* (yellow snakes) from one clutch (mean ± SEM: mass =52.81±2.87 g, range 43.6-69.25 g; SVL =512.5±6.48 mm, range 480-530 mm) (2 controls died of neurological problems after the treatment period and before the behavioral testing). Ten snakes were captive born *Elaphe obsoleta obsoleta* (black snakes) from another clutch (mean ± SEM: mass =63.9 g, range 42.9-83.04 g; SVL =524 mm, range 480-550). Although both clutches were born in captivity, the yellow snakes were hatched from long term captives of the UT Veterinary School (originally from FL), and the black snakes were hatched from wild caught adults from Knox County, TN. These groups were not significantly different in size as revealed by a MANOVA [treatment: ?=0.40, $F(2,12)=0.247$, $p=0.785$, mass: $F(1,12)=0.193$, $p=0.667$, SVL: $F(1,12)=0.488$, $p=0.497$; clutch ?=2.65, $F(2,12)=2.158$, $p=0.158$, mass: $F(1,12)=4.649$, $p=0.050$, SVL: $F(1,12)=2.721$, $p=0.123$].

Housing:

The yellow snakes were housed individually in an acrylic cage (Figure 2.1; patent: Waters et al., 1999), divided in half with a white acrylic panel, containing a water dish, hide box, and a rough brick (to assist in shedding). The enclosures measured 30 x 50 x 40 cm. The black snakes were housed in fiberglass kennels measuring 40 x 60 x 50 cm. The kennels had screen doors with clear acrylic frames and were divided with a white acrylic panel. (Note: during behavioral analyses, the black snakes were housed in the enclosures described above for yellow snakes.) All snakes were housed in the housing room, which was maintained on a 12-hour light/dark cycle with an ambient temperature of 27-30 °C.

The snakes were randomly assigned to groups regardless of sex. Nine subjects (five male and four female) were housed in environmentally enriched conditions

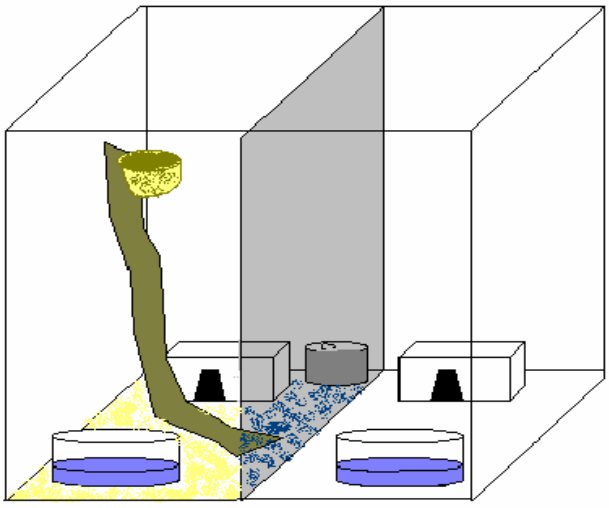

Figure 2.1: Photograph and schematic representation of the differential housing for enriched and control snakes. Each compartment measured 30 x 50 x 40 cm.

(structurally complex) and fed live prey weekly (EC, enriched condition). Enriched cages contained aspen bedding as a substrate, a branch for vertical locomotion, a half coconut on top of the branch to simulate a cavity in a tree, and a plastic container filled with moist sphagnum moss. The enriched enclosures were designed to be as "natural" as possible and thus contained a simulated tree for climbing in addition to substrate for burrowing. Furthermore, they were provided live food as both stimulus objects and as representative prey that would be eaten in the wild. Nine control snakes (five male and four female) were housed in standard laboratory conditions (structurally simple) and fed dead prey weekly (IC, impoverished condition). Standard cages were lined with corrugated paper substrate and had no vertical climbing object. The impoverished condition was representative of many standard laboratory cages: no stimulus objects and dead prey, which are not typically eaten in the wild.

The snakes were housed for eight months in their appropriate conditions before being measured and behaviorally tested (though they remained in their respective housing throughout the behavioral analysis; Figure 2.2).

Prey item used and feeding regime:

Each snake was fed one fuzzy mouse weekly (*Mus musculus*). All prey fed on one day were from the same litter and their weights ranged from 6-8 grams during the

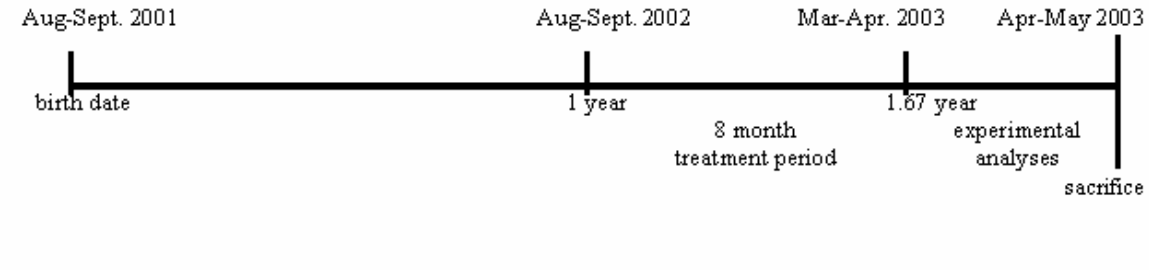

Figure 2.2: Experimental procedure timeline

course of the study. The mice were kept at the University of Tennessee Veterinary School, and the prey that needed to be euthanized were done so with CO_2 suffocation. The dead prey were feed immediately to the snakes or frozen for a later feeding (see Table A.1 for morphology and meal data).

Statistical analyses:

For the majority of the experiments, I performed multivariate analyses of covariance (MANCOVA) and repeated measures ANOVAs. The MANCOVA is a conservative test of a treatment effect and accounts for correlated multiple response variables (Tabachnick & Fidell, 1989). Except for the repeated measures component, I only statistically tested the main effects of the response variables. Statistically testing the interactions reduced the power of my design to levels that could not reveal differences between the groups even if one truly existed. Significance of multivariate results after variance from the covariate was removed from the error variance was evaluated with Wilks' Lambda statistic. When significant, I then performed univariate ANOVAs on each of the response variables. Univariate ANOVAs for each variable served as a tool in interpreting the results of the MANOVA; i.e., they aided in the assessment of which variable(s) may have contributed to a significant multivariate response (Tabachnick & Fidell, 1989).

A mixed design repeated measure ANOVA was used when multiple observations were made on the same subject; treatment and clutch were used as between-subjects factors and time (e.g., day, trial) was used as a within-subjects repeated measure. As my data in general did not have problems with sphericity, I followed the univariate approach, which considers the dependent variables as responses to the levels of within-subjects factors. Thus both the "within-subjects effects" and the "between-subjects effects" are reported as univariate ANOVAs.

When a specific task required a different type of analysis than was listed above, it was described in the corresponding section. Analyses with clutch as the independent variable were analyzed as two-tailed tests; however, the directional nature of the predicted treatment effects on the dependent variables supported use of one-tailed tests.

Analyses were designated as significant at p-values less than 0.05. All analyses for this project were conducted using SPSS 11.5 (SPSS Inc., 1989-2002).

Part 2 Morphological study

To determine if differential experience in housing and feeding altered body morphology, changes in specific head and body measurements were investigated.

Materials and methods:

Before and after the eight month treatment period, I took standard measurements of snout-vent length, tail length, and mass. Before conducting the behavioral assays, I took cranial morphological measurements (as per Bonnet et al., 2001; Krause et al., in press) on head width, jaw length, interocular distance, and eye width with Mitutoyo digimatic calipers. The snakes were each measured once and then the procedure was repeated twice more without any reference to previous measurements.

I performed a MANCOVA, using grams of prey consumed as a covariate, to test whether housing condition (enriched and standard) affected mass and snout-vent length. If the MANCOVA revealed a significant overall effect, I then performed univariate ANCOVAs on each of the response variables. I also performed a MANCOVA, with snout-vent length as a covariate, to test whether housing condition affected head measurements. In addition, I looked at clutch and individual differences in the morphological head and body measurements.

Part 3 Feeding study

This study examined the foraging efficiency of rat snakes that had experience with only one type of prey for most of their sub-adult life. (Note, after hatching, all snakes were fed live pinkie mice for approximately the first two months and then were fed dead prey for the remainder of the year.) As such, the IC snakes did not have any opportunities to perfect their prey handling skills with live mice.

Materials and methods:

The feeding studies followed an A-B-A-B design with "A" being familiar prey and "B" being unfamiliar prey. The snakes first received the familiar prey and then 10 days later the snakes received the opposite prey type. This procedure was then repeated at subsequent 10 day intervals so that each snake received two trials with dead prey and two trials with live prey. Prior observations with weekly feedings demonstrated preferred feeding times for the yellow snakes and the black snakes (evening and mid-day, respectively). Furthermore, the yellow snakes would not reliably feed in front of an observer or camera, thus their feeding trials were conducted in the dark between 19:00 and 22:00 hours in their home cages. The feeding trials for the black snakes were conducted in the light between 12:00 and 15:00 hours in their home cages. The testing room (i.e., housing room) was maintained at approximately 28 °C during these feeding sessions.

Each trial began with the introduction of a live or dead mouse (mice were euthanized and fed immediately unlike weekly feedings). If the snake did not consume the mouse within 30 minutes, the trial was terminated and repeated three days later. All trials were recorded with an 8mm camcorder and scored with the Observer software. Trials for the yellow snakes were recorded with a camera equipped with IR lights and detection. [The snakes seem to be unable to detect IR wavelengths (personal observation; P. Andreadis, unpublished observation).] Testing variables were modeled after those of Halloy & Burghardt (1990), Mori (1996), Krause & Burghardt (2001), and Mehta (in press): condition of prey (live or dead), capture position (anterior, middle, posterior), prey-handling method (simple seizing, pinion, constriction), type of coil (regular or irregular, if constriction was present), prey position at ingestion (anterior, middle, posterior), feeding proficiency (number of missed attempts, "unsuccessful handling" time, handling time, and swallowing time), and total feeding duration (see Appendix B for definitions).

Chi square analyses were used to determine differences in the categorical responses (i.e., capture position, prey handling method, and prey position at ingestion). I performed a mixed design 3x1 repeated measures ANOVA to determine the effect of

treatment (enriched and standard housing condition), clutch, and prey type on the feeding proficiency behaviors. Trial was the repeated measures factor.

Part 4 Exploratory study

To determine if snakes housed in enriched environments exhibited increased exploratory behavior in an open field task, the number of tongue flicks, number of grid crosses, and amount of escape-rearing were measured. Due to the literature on studies with rodents, I expected that snakes raised in enriched environments would initially explore more (as evidenced by increased tongue flicking and grid crossing) but would habituate more quickly within each trial and in subsequent exposures to the open field apparatus.

Materials and methods:

The open field was a box (95 cm x 95 cm x 60 cm) constructed of plywood and painted black. The floor of the apparatus was lined with a corrugated plastic sheet cut to fit snugly against the wall. I mounted one camera on the ceiling above the open-field (to score horizontal movement) and used a hand-held camera to zoom in on the snake (for counts of rearing and tongue flicks). I conducted the trials at dusk (between 16:00 and 19:00 hours) in a dark room and recorded with cameras equipped with IR lights and detection.

The trials were conducted in a testing room (ambient temperature at 28 °C) located adjacent to the housing room. I carried the snake by hand into the testing room, placed it in the center of the apparatus, and allowed it approximately 60 seconds to acclimate. I began each trial with a verbal start cue to coordinate the hand-held and overhead cameras. A trial lasted for 10 minutes after which the snake was returned to its home cage. After each trial, I thoroughly washed the apparatus with a mild odorless detergent and dried it with paper towels. The snakes were tested in alternating order (e.g., enriched then control) once a day for three consecutive days.

To minimize experimenter interference, I video-recorded all trials and then analyzed the tapes with the Observer software. I reviewed the tapes after all of the

experiments were conducted. I divided the monitor into 25 squares (to count grid crossings) and scored these behaviors: tongue flicks, ambulation (measure by number of grid crosses), number of bouts of rearing, and time spent rearing.

A mixed design 2x2 repeated measures ANOVA was used to determine the effect of treatment (enriched and standard housing condition) and clutch on the three response variables (tongue flicks, ambulation, and rearing). Trial and minutes per trial were the repeated measure factors. Regression lines were plotted for tongue flicks and grid crossings, and the slope and y-intercept were determined for each trial on each subject. These measures are important because the intercept provides a rough estimate of general responsiveness and slope corresponds to habituation rate (see Bowers, 1992 for discussion). A 2x2 repeated measures ANOVA was used to determine treatment and clutch effects on the slope and y-intercepts of the exploratory behaviors. An independent samples t-test was conducted to determine a treatment effect on the difference scores between trials 1 and 3 for the number of tongue flicks and grid crossings.

Part 5 Learning study

This study examined potential differences in learning ability due to housing condition. I hypothesized that due to their experience with a more stimulating environment, the EC snakes would be better able to learn this task for a "food" or escape reward.

Materials and methods:

The testing apparatus (see Figure 2.3) consisted of a circular platform (150 cm diameter) with 12 holes (each 5 cm in diameter) equally positioned around the perimeter (holes were 6 cm from edge). One goal hole was randomly chosen for each snake and led to a dark refuge, filled with moist paper towels, attached under the platform; the other 11 holes were rendered inaccessible with a plastic card taped beneath the platform. A clear acrylic barrier (30 cm tall) was attached to the outside of the platform to permit unobstructed observation. As per Holtzman's protocol (1999), the apparatus was illuminated with six 150 watt spot bulbs (for extreme heat and bright light) to elicit an

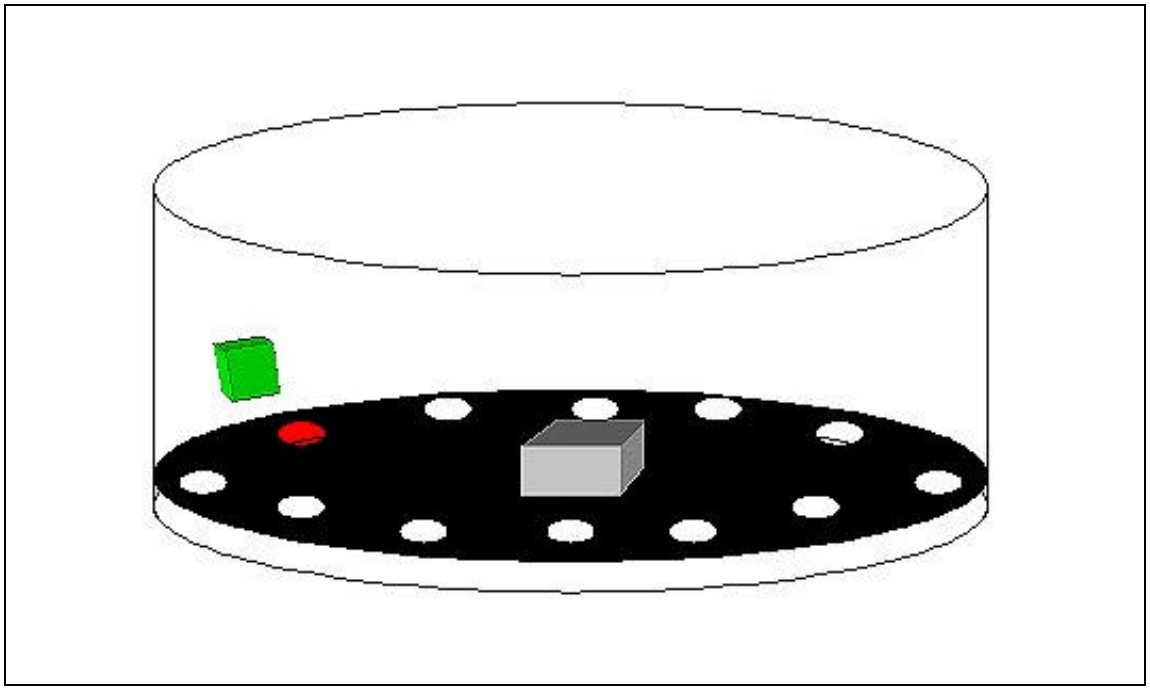

Figure 2.3: Experimental test arena (150 cm) for learning studies. The apparatus consisted on a wooden platform, painted black, with 12 holes (each 5 cm in diameter) drilled 6 cm from the periphery and a clear acrylic barrier around edge (30 cm tall). Representative goal is labeled in red with intramaze cue (green square) above it and a refuge hide box underneath (not shown). The walls of the testing room were differentially patterned to provide distal cues (not shown). To start the trial the hide box in center was removed after 30 sec.

escape response from the snakes (the area immediately above and on the apparatus reached temperatures around 32-34 °C). I provided distal and proximal visual cues to allow orientation to the escape hole. Distal cues (outside the apparatus) included differentially patterned walls in the testing room (plain black wall, plain white wall, wall with black vertical stripes, and wall with a door). Proximal cues (inside the apparatus) included a green square placed directly behind the escape hole. Odors from the hide box under the escape hole may also have provided a proximal cue.

The trials were conducted in a testing room (ambient temperature at 28 °C) located adjacent to the housing room. I carried the snake by hand into the testing room, placed it in the center of the apparatus, and allowed it approximately 60 seconds to acclimate. A trial lasted for 10 minutes or until the escape hole was found. After each trial, I thoroughly washed the apparatus with a mild odorless detergent and dried it with paper towels (monitoring the next trial to make sure that the current snake did not follow the path of the previous snake). I tested the snakes in alternating order (e.g., enriched then control) three times/day for two consecutive days and then repeated that design two weeks later. The first trial on each day was always a control: there was no odor cue available. The second and third trials on the first and third days had an odor trial: while the snake was in the holding box, I rubbed a dead mouse from the center of the apparatus directly to the assigned goal hole. On the second and fourth days, the second and third trials had odor from a mouse in the goal box (wet paper towels that had been wrapped around a dead mouse). In any trial, if the snake did not find the goal hole, it was gently prodded to the hole and allowed to remain there for 1.5 minutes before being removed from the apparatus.

To minimize experimenter interference, I video-recorded all trials with an overhead camera and reviewed the tapes after all of the experiments are conducted. The apparatus was divided into four quadrants when seen on the monitor screen. These behaviors were scored and analyzed with the Observer software: latency to reach goal hole, latency and time spent in goal quadrant, and number of errors.

I performed a mixed design 2x2 repeated measures ANOVA to determine the effect of treatment (enriched and standard housing condition) and clutch on the four

response variables (latency to reach goal hole, number of errors, latency to reach goal quadrant, and time spent in goal quadrant). Day and trial were the repeated measures factors.

Chapter 3: RESULTS

Part 1 Morphological study

Body measurements:

Descriptive statistics for body measurements are shown in Table A.1 and Table 3.1. Comparisons of body size (mass, snout-vent length (SVL), and growth in each variable) were assessed with a MANCOVA with grams of food consumed as a covariate [Table A.3; ?=0.236, F(4,9)=7.274, p=0.007]. The analysis revealed significant differences in housing treatment [?=0.441, F(4,9)=2.851, p=0.022] and clutch [?=0.219, F(4,9)=8.038, p=0.005], with EC and black snakes being larger than IC and yellow snakes respectively.

The corresponding univariate analysis yielded significant differences in mass, snout-vent length (SVL), and growth rates for housing treatment and significant differences in mass and growth in mass for clutch (Table A.4). For housing treatment effects, the EC snakes were larger [Figure 3.1; mass: F(1,12)=6.060, p=0.025; SVL: F(1,12)=3.433, p=0.045] and had increased growth rates [Figure 3.2; mass growth: F(1,12)=7.051, p=0.011; SVL growth: F(1,12)=4.815,p=0.024]. For clutch effects, black snakes were larger in mass but not SVL [Figure 3.3A, mass: F(1,12)=16.616, p=0.001, SVL: F(1,12)=2.042, p=0.117]. Furthermore, the rate of growth in mass between clutches was also significantly different [Figure 3.3B; F(1,12)=20.609, p=0.001].

Table 3.1 Morphometry data shown as mean ± standard error of the mean.

Group	N	Mass (g)	Mass growth (g)	SVL (mm)	SVL growth (mm)
control	7	98.2 ± 7.2	45.7 ± 5.3	685.5 ± 16.3	174.9 ± 7.9
enriched	9	124.4 ± 5.8	66.9 ± 6.7	739.3 ± 13.1	220.9 ± 11.1
yellow	8	93.8 ± 5.9	45.7 ± 3.9	699.8 ± 14.0	198.8 ± 17.6
black	10	129.2 ± 4.5	64.8 ± 6.9	726.9 ± 10.6	202.0 ± 10.8

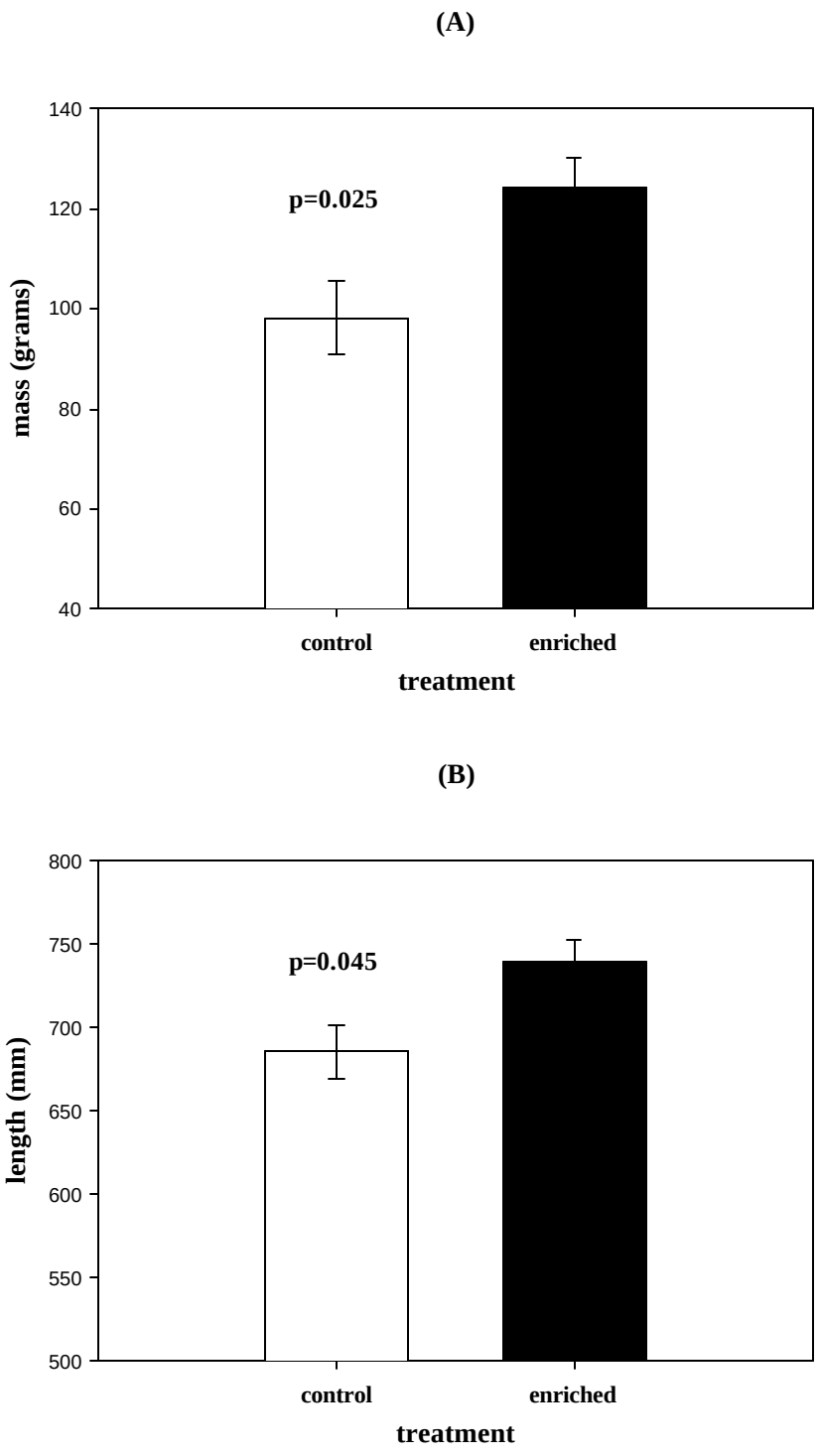

Figure 3.1: Effect of housing treatment group on mass and snout-vent length (SVL). (A) Mass differences between treatment groups, (B) SVL differences between treatment groups. Shown are means +/- the standard error of the mean.

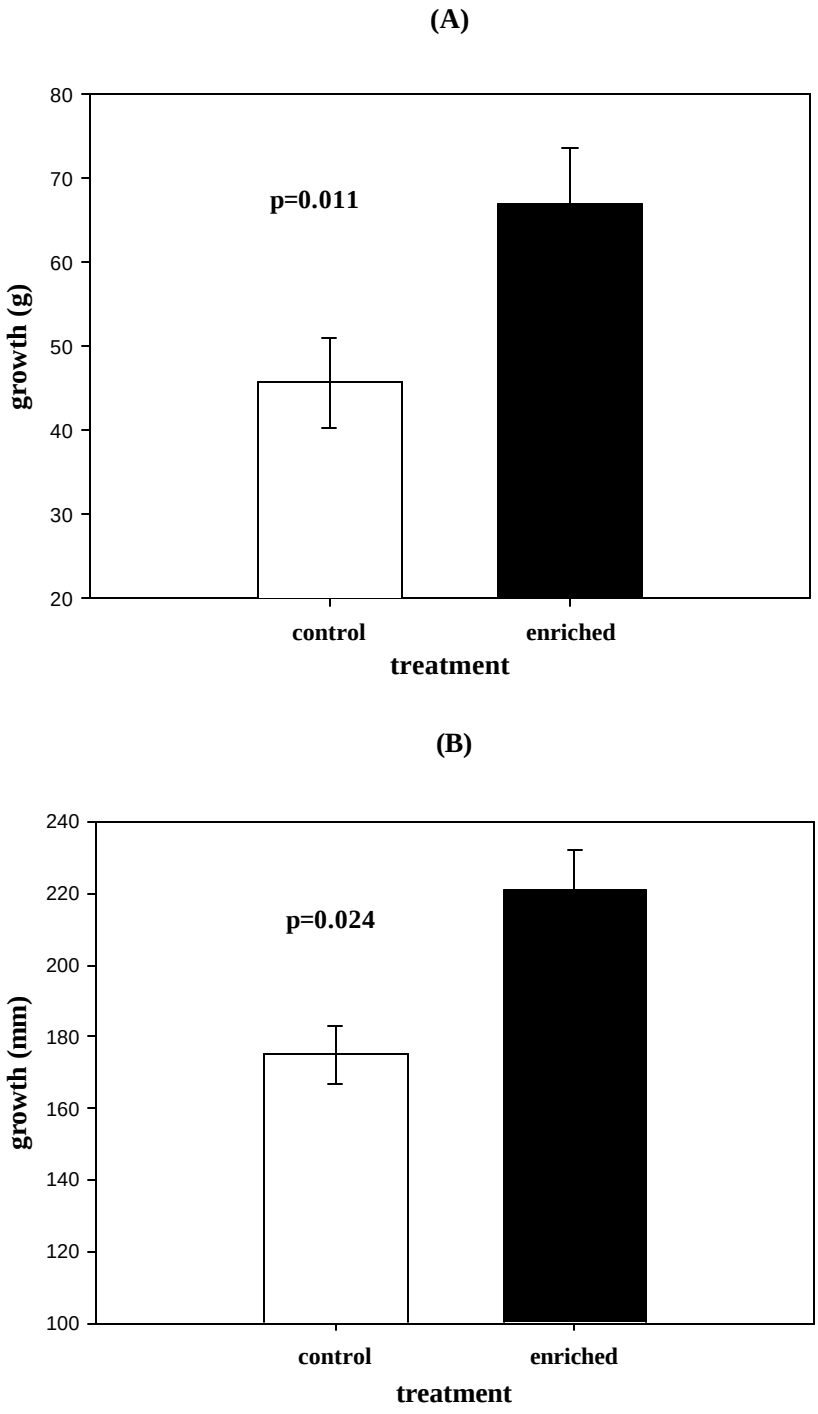

Figure 3.2: Effect of housing treatment group on the growth in mass and snout-vent length (SVL). (A) Growth differences in mass between treatment groups, (B) Growth differences in SVL between treatment groups. Shown are means +/- the standard error of the mean.

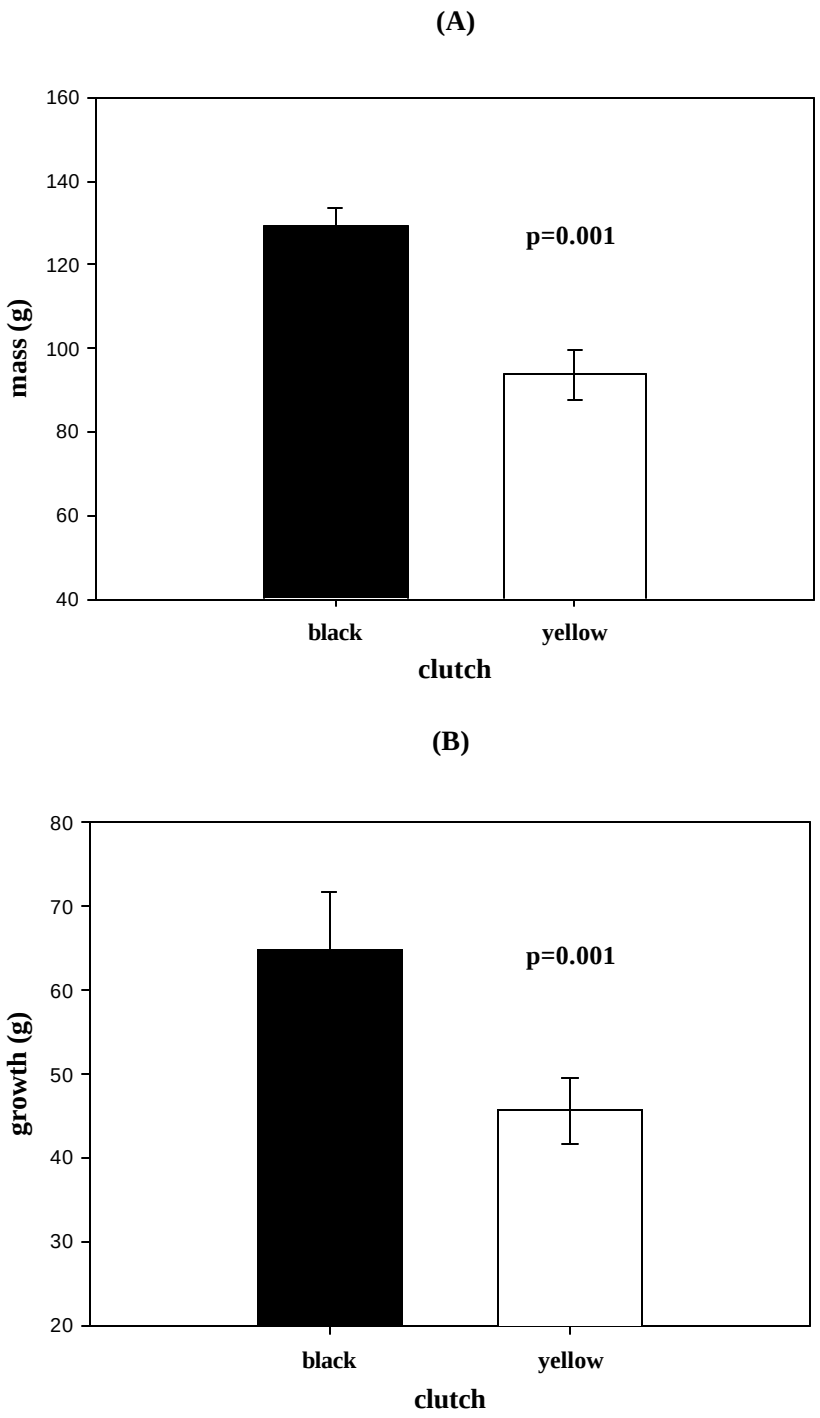

Figure 3.3: Effect of clutch on mass and growth in mass. (A) Mass differences between clutches, (B) Growth differences in mass between clutches. Shown are means +/- the standard error of the mean.

Table 3.2 Head measurements shown as means ± standard errors of the mean.

Group	Head length (mm)	Head width (mm)	Jaw length (mm)	Interocular distance (mm)	Eye diameter (mm)
control	17.9 ± 0.2	13.4 ± 0.3	19.4 ± 0.4	9.6 ± 0.2	3.9 ± 0.1
enriched	18.6 ± 0.3	13.6 ± 0.3	19.7 ± 0.4	9.6 ± 0.2	3.8 ± 0.1
yellow	17.8 ± 0.4	13.9 ± 0.2	18.5 ± 0.3	9.8 ± 0.3	3.6 ± 0.1
black	18.6 ± 0.2	13.3 ± 0.3	20.2 ± 0.2	9.5 ± 0.1	3.9 ± 0.05

Head measurements:

Descriptive statistics for head measurements are shown in Table A.3 and Table 3.2. Since snout-vent length marginally co-varied with head measurements, it was kept in the MANCOVA (Table A.5). The analysis revealed significant differences in clutch [?=0.174, F(5,7)=6.660, p=0.006] for the overall head measurements but not in treatment [?=0.110, F(5,7)=0.174, p=0.482].

Univariate analysis revealed significant (or marginally significant) differences in head length, head width, jaw length, and eye diameter (Table A.6; Figure 3.4) with black snakes having increased head length, jaw length, and eye diameter as compared to yellow snakes.

Part 2 Feeding study

Out of 34 feeding trials that resulted in constriction, eight trials involved "irregular" coils (see Appendix 1 for definition) and only one "irregular coil" was performed by an EC snake; thus, 87.5% of irregular coiling patterns were conducted by IC snakes. Pearson chi-square analyses were used for the categorical data (prey capture position, prey ingestion position, and prey handling method; Table A.7) to test for differences between clutch and housing treatment groups (Table A.8-10). The tests revealed no significant differences in clutch or treatment in any of the measurements, though trends are shown in Figure 3.5. IC snakes tended to capture more prey tail first and were the only snakes to capture dead prey tail first. Following these captures, IC snakes ingested more prey tail first, which tends to slow down the ingestion process.

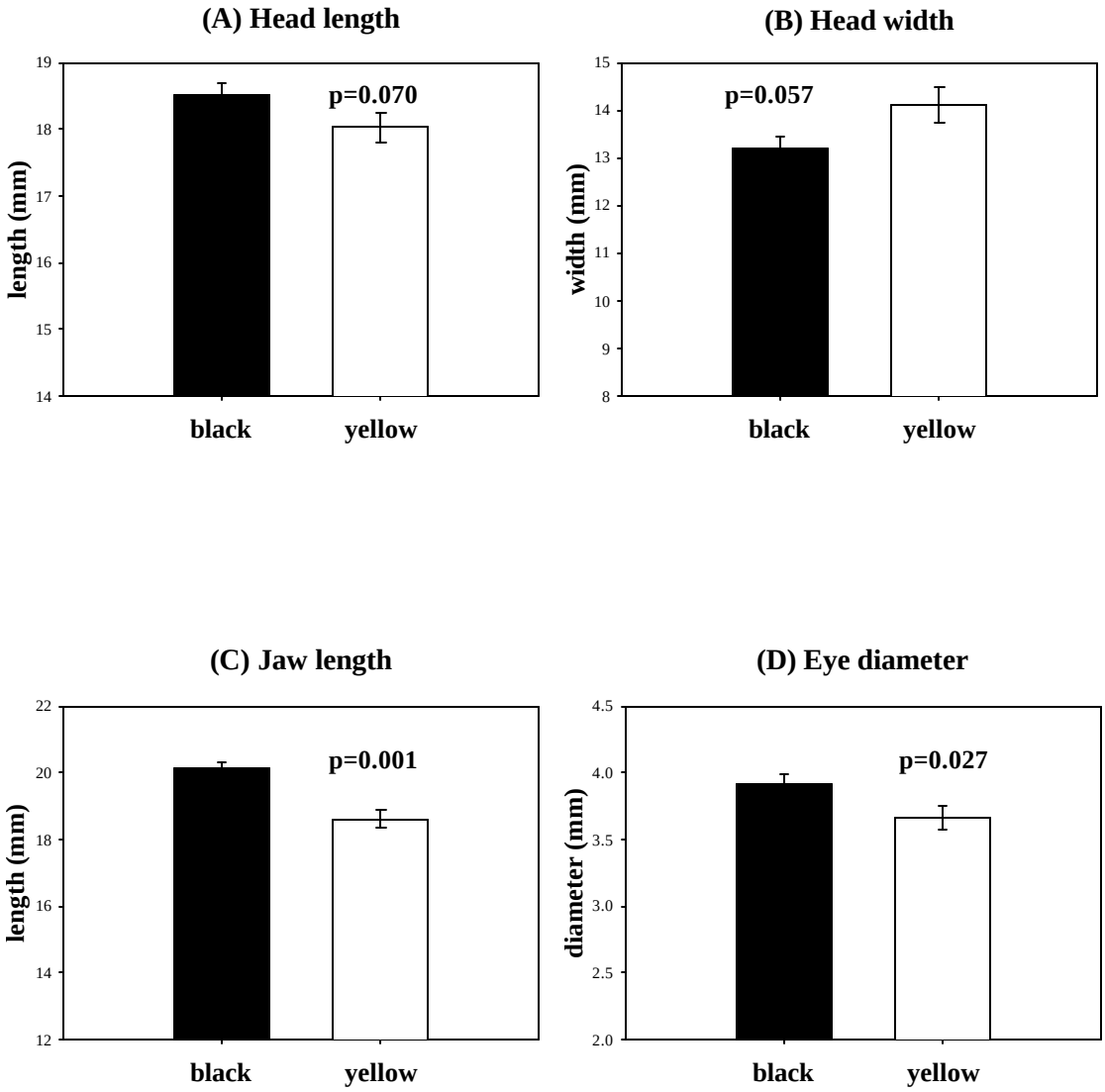

Figure 3.4: Head morphology differences between black and yellow snakes. (A) Head length, (B) Head width, (C) Jaw length, and (D) Eye diameter, of black and yellow snakes. Shown are means +/- the standard error of the mean.

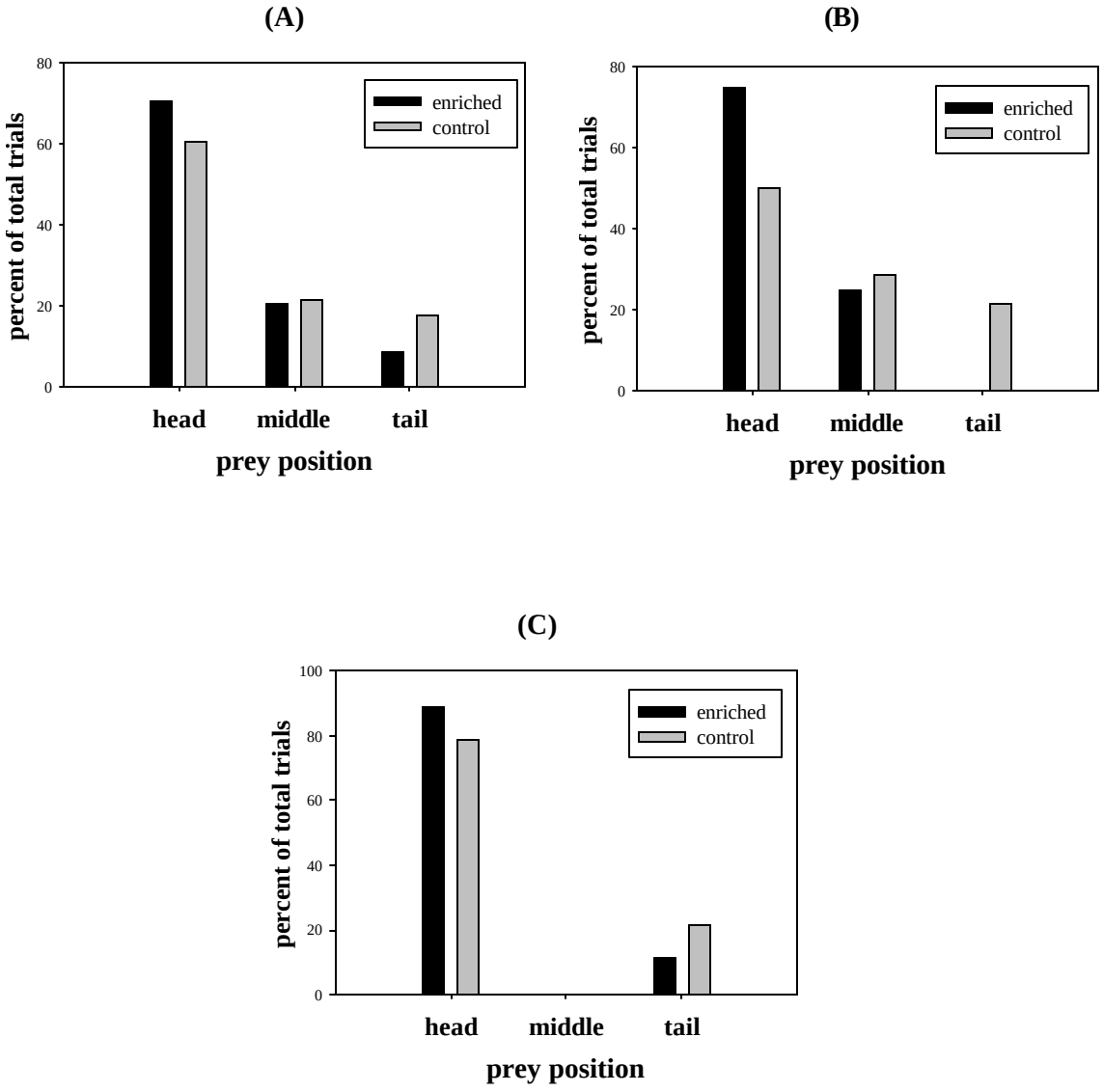

Figure 3.5: Effect of housing treatment group on the position of prey at capture and ingestion. (A) Prey capture position for all prey, (B) Prey capture position with dead prey only, (C) Ingestion position of live prey only.

Table 3.3 Times and effect of prey type on the various feeding behaviors. Data are shown as mean ± standard error of the mean.

Factor	N	Number of missed attempts	Handling time (s)	Swallowing time (s)	Mishandling time (s)	Consumption time (s)	Total trial time(s)
control	7	0.9 ± 0.4	114.4 ± 17.2	202.8 ± 15.7	54.6 ± 22.7	362.2 ± 28.3	371.8 ± 41.3
enriched	9	1.6 ± 0.4	90.3 ± 14.9	188.5 ± 13.7	71.9 ± 19.8	278.4 ± 24.6	350.8 ± 35.9
yellow	8	1.4 ± 0.5	84.2 ± 18.1	151.9 ± 16.6	56.2 ± 24.0	243.7 ± 29.9	292.4 ± 43.6
black	10	1.1 ± 0.4	120.5 ± 14.1	239.4 ± 12.9	70.3 ± 18.7	396.8 ± 23.3	430.2 ± 33.9
dead prey	15	0.6 ± 0.4	57.4 ± 16.1	207.4 ± 14.7	27.1 ± 21.3	293.1 ± 26.5	291.9 ± 38.7
live prey	16	1.9 ± 0.4	147.3 ± 15.8	183.9 ± 14.4	99.4 ± 20.9	347.5 ± 25.9	430.7 ± 37.9

A repeated measures design was used for all other time and count data. Each prey type (dead or live) had two trials, creating the repeated measures component. The results are depicted in Table 3.3 and are explained below.

Missed attempts:

Subjects missed live prey more than dead prey (Figure 3.6A) and there was a significant main effect of prey type [$F(1,27)=5.323$, $p=0.029$]. However, there were no significant differences across trials (Table A.11).

Unsuccessful handling time:

There was significant main effect of prey type (Figure 3.6B) on unsuccessful handling time: subjects spent more time handling live prey, which did not end in successful capture or ingestion [$F(1,27)=6.090$, $p=0.020$]. There were no significant differences between housing treatment groups or across trials (Table A.12).

Handling time:

There was a significant main effect of prey type (Figure 3.6C) on handling time: subjects spent more time handling live prey than dead prey [$F(1,27)=17.529$, $p=0.000$].

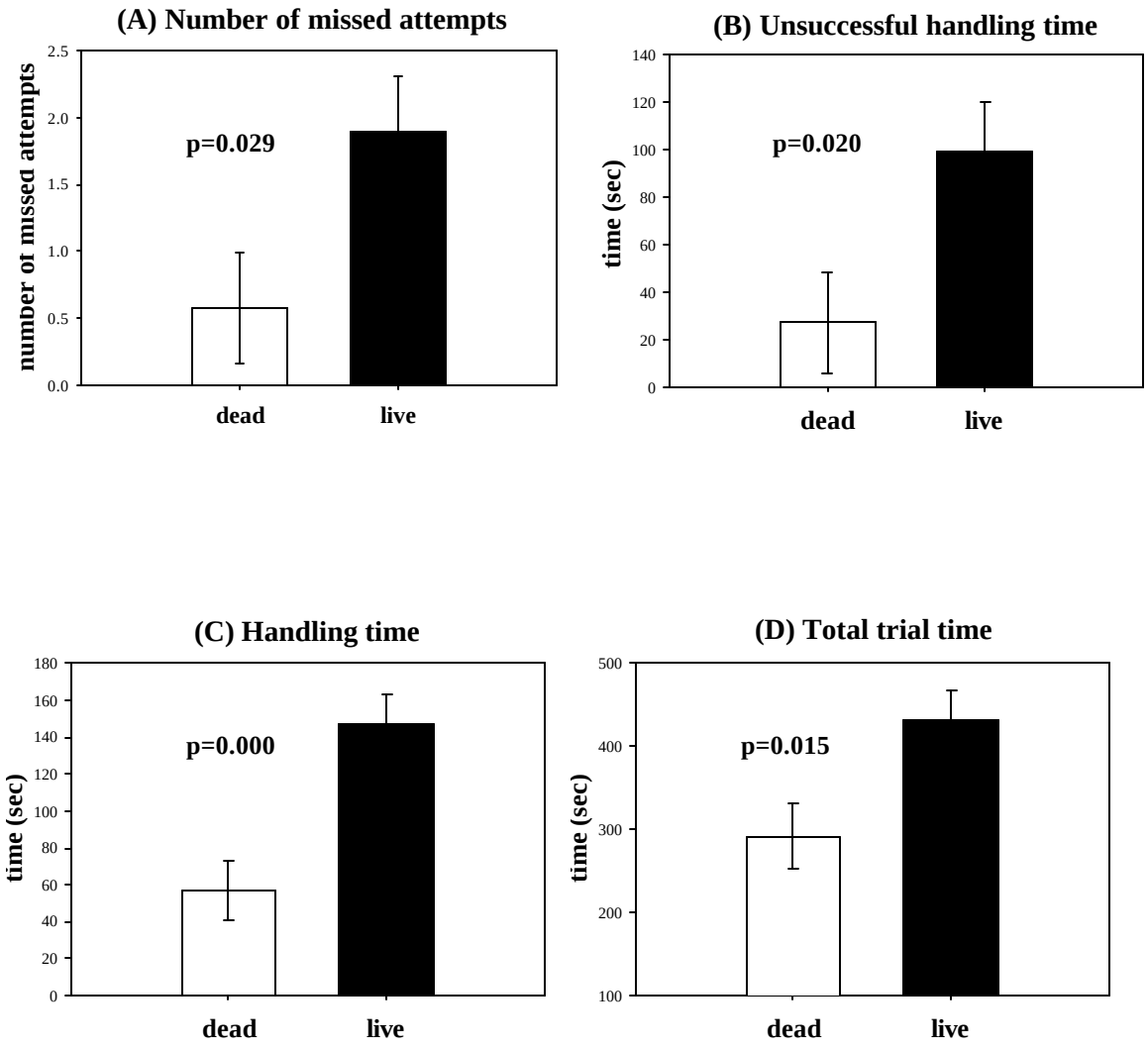

Figure 3.6: Effect of prey type (live versus dead) on certain feeding measures. (A) The number of missed attempts, (B) Unsuccessful handling time, (C) Handling time, (D) Total trial time, when feeding on live versus dead prey. Shown are means +/- the standard error of the mean.

There were no significant differences between housing treatment groups or clutches (Table A.13). A significant main effect of handling time across trials revealed that the subjects actually had longer handling times on the second trial (trial 1 M=75.3 ± 8.3 sec, trial 2 M=129.4 ± 20.5 sec).

Swallowing time:

There was a significant main effect of clutch [F(1,27)=17.057, p=0.000] with yellow snakes having shorter swallowing times. There were no significant differences between housing treatment groups or across trials for swallowing time (Table A.14).

Total consumption time:

There was a significant main effect of treatment [F(1,27)=5.059, p=0.017] and clutch [F(1,27)=16.182, p=0.000] in total consumption time (i.e., feeding behaviors that actually culminated in ingestion; sum of handling and swallowing times; Table A.15, Figure 3.7). Similar to the handling time measurement, the second trial was also significantly longer than the first for this measurement (trial 1 M=288.2 ± 19.0 sec, trial 2 M=352.4 ± 24.6 sec).

Total trial time:

Total trial time includes both the successful and unsuccessful components of feeding (i.e., unsuccessful handling, handling, and swallowing time). There were significant main effects of clutch [F(1,27)=6.154, p=0.020] and prey type (Figure 3.6D; [F(1,27)=6.806, p=0.014]) with black snakes and dead prey incurring longer total trial times, but there were no differences between housing treatment groups (Table A.16).

Part 3 Exploratory study

Tongue flicks:

Descriptive statistics are presented in Table 3.4. When minutes were included as a repeated variable in the analysis, a repeated measures ANOVA on the number of

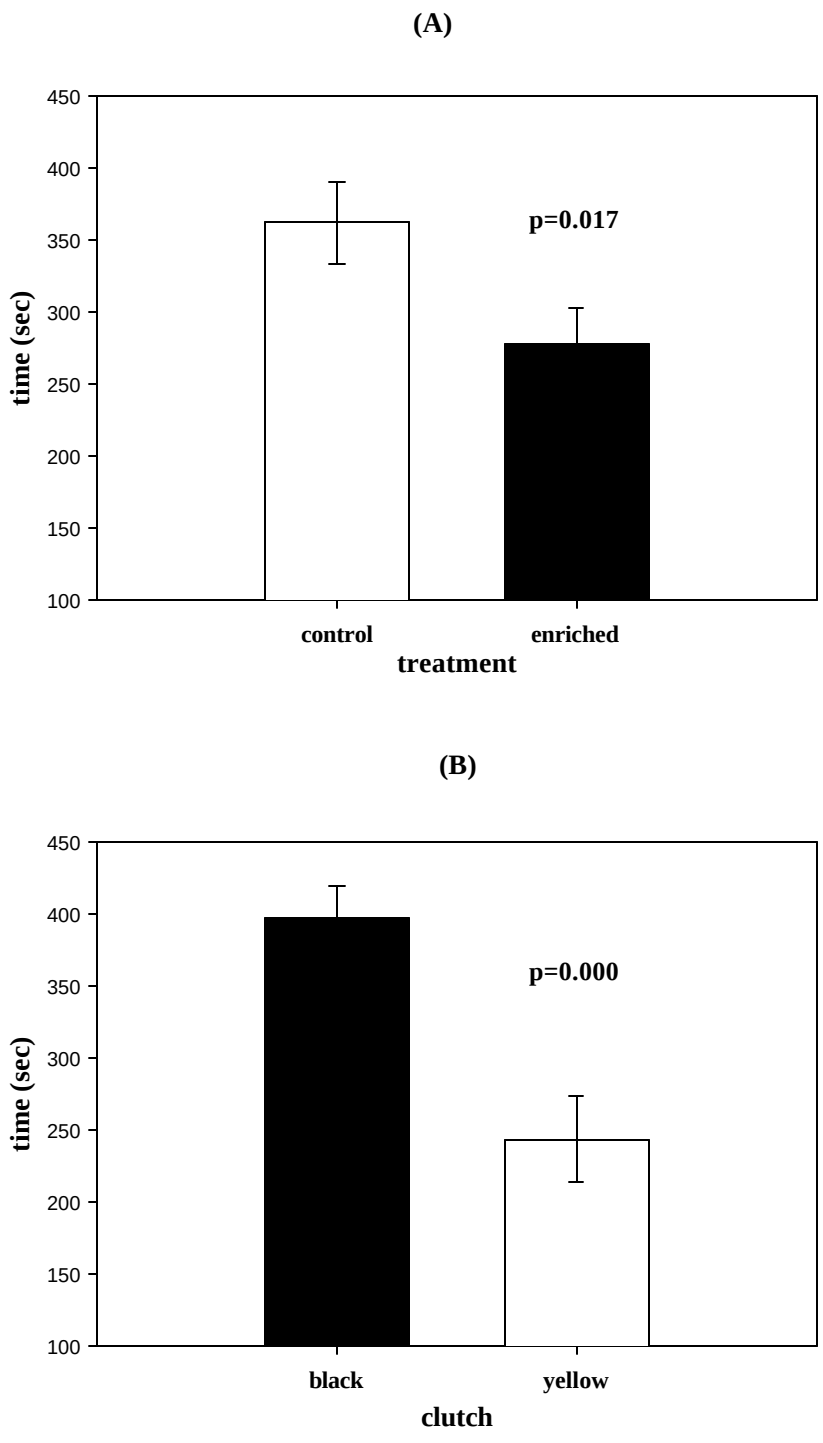

Figure 3.7: Total consumption time (prey handling time plus swallowing time). (A) Total consumption time by housing treatment, (B) Total consumption time by clutch. Shown are means +/- the standard error of the mean.

Table 3.4 Tongue flick counts per trial. Data shown are means ± standard errors of the mean.

Group	N	TRIAL 1	TRIAL 2	TRIAL 3
control	7	599.1 ± 44.2	527.4 ± 92.8	699.7 ± 40.5
enriched	9	651.7 ± 39.7	690.4 ± 38.6	616.4 ±51.7
yellow	8	681.2 ± 30.7	632.2 ± 41.2	610.3 ± 63.2
black	10	597.2 ±41.0	611.3 ± 76.1	678.4 ± 41.0

tongue flicks revealed no significant between-subjects effects (Table A.17). There was a significant interaction of trial by treatment with EC snakes having higher initial rates of tongue flicking than IC snakes but then showing a marked habituation on the last trial [Figure 3.8A; $F(2,26)=3.469$, $p=0.023$]. There was also a marginal significance across minutes [Figure 3.9A; $F(9,117)=1.854$, $p=0.066$] showing an overall decrease in tongue flick rates by all groups combined.

In order to assess whether the tongue flicking response habituated over the minutes in each trial, individual regressions were conducted. There were no significant differences in either the slope or y-intercept (Table A.19). Within trials, averaged slope data demonstrated a slight habituation trend within trials by IC but not EC snakes (mean slope ± SEM: control M= -1.15 ± 0.41; enriched M= -0.006 ± 0.46).

To assess habituation in the tongue flick response across trials, t-tests were conducted on the difference in the scores on trial 1 subtracted by the scores on trial 3. This analysis revealed habituation by EC snakes and an increase in response by IC snakes [Table A.21; $t(14)=1.847$, $p=0.043$, mean counts ± SEM: EC M= 35.2 ± 37.7, IC M= -100.6 ± 68.4].

Number of grids crossed:

Descriptive statistics are presented in Table 3.5. A repeated measures ANOVA on the number of grids crossed revealed no significant between-subjects effects (Table A.19). There was a significant interaction of trial by treatment, with EC snakes

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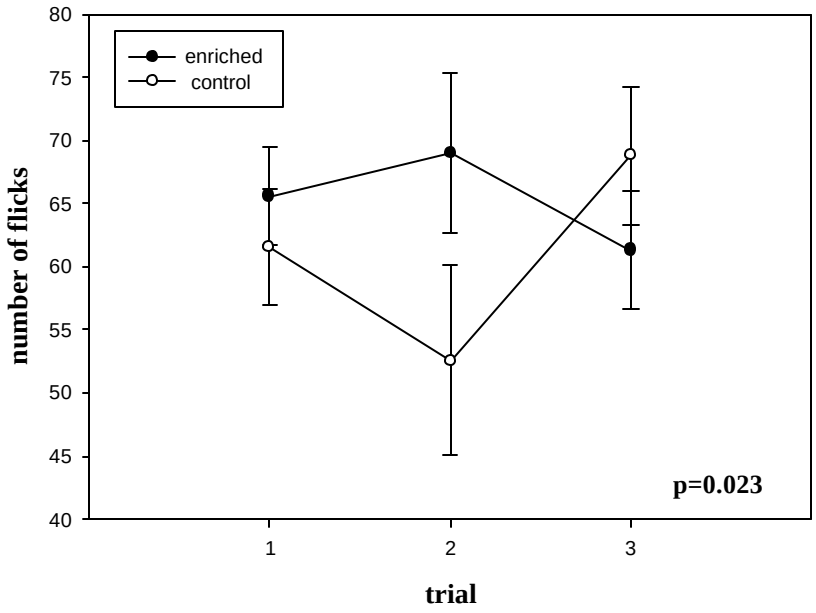

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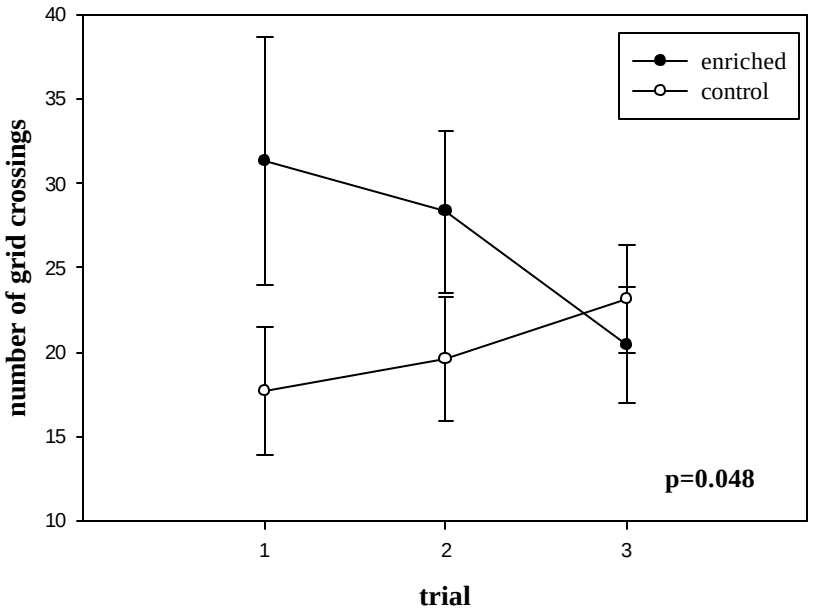
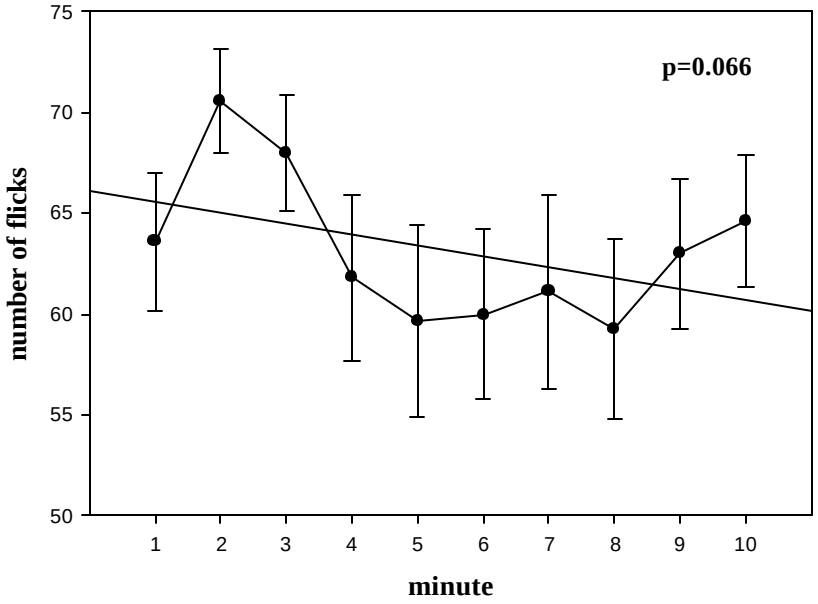


Figure 3.8: Number of tongue flicks and grid crossings per trial. (A) Number of tongue flicks per trial, (B) Number of grid crossings per trial. Shown are means +/- the standard error of the mean.

(A)



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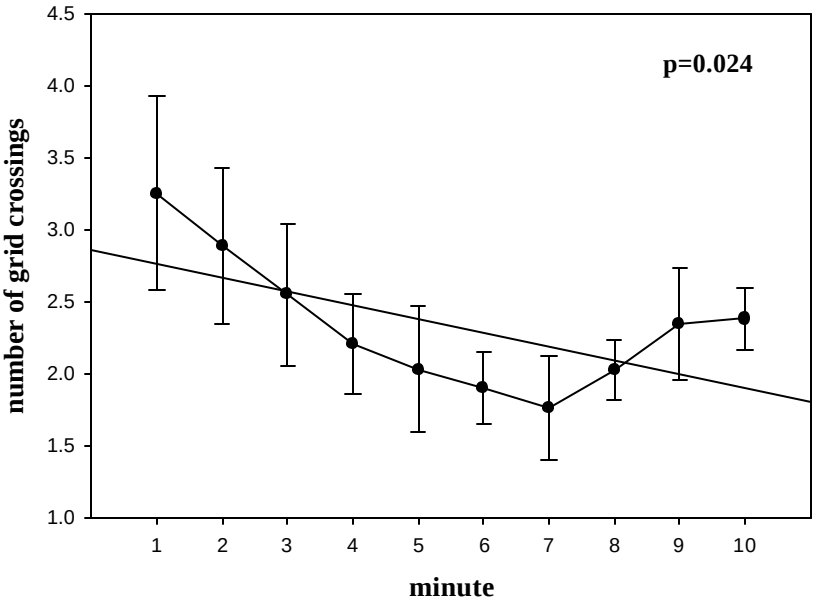

Figure 3.9: Number of tongue flicks and grid crossings averaged across trials for all subjects combined. Regression lines show habituation in both measures. Shown are means +/- the standard error of the mean.

Table 3.5 Numbers of grid crossings per trial. Data shown are means ± standard errors of the mean.

Group	N	TRIAL 1	TRIAL 2	TRIAL 3
control	7	17.7 ± 3.8	19.6 ± 3.7	23.1 ± 3.2
enriched	9	31.3 ± 7.4	28.3 ± 4.8	20.4 ± 3.4
yellow	8	21.2 ± 5.1	21.5 ± 1.9	19.8 ± 2.6
black	10	27.9 ± 6.9	26.3 ± 5.1	22.7 ± 3.4

habituating over the trials as compared to IC snakes [Figure 3.8A; F(2,26)=2.570, p=0.048]. Similar to the tongue flick data, there was a significant main effect of minute within subjects [Figure 3.9B; F(9,117)=2.234, p=0.024].

Regression data revealed no significant difference in either the slope or y-intercept (Table A.20). Therefore, neither group alone demonstrated significant habituation throughout the minutes of each trial.

T-tests on the difference scores revealed habituation by EC snakes and an increase in response by IC snakes across trials [Table A.21; t(14)=1.850, p=0.043, EC M= 10.9 ± 6.9, IC M= -5.4 ± -4.6].

Number of escape-rear bouts:

Descriptive statistics are presented in Table 3.6. A repeated measures ANOVA on the number of escape rear bouts revealed no significant between-subjects effects [housing treatment: F(1,13)=1.822, p=0.100] or within-subjects effects [trial: F(2,26)=0.203, p=0.817; minute: F(9,117)=0.748, p=0.665)].

Time spent escape-rearing:

Descriptive statistics are presented in Table 3.7. A repeated measures ANOVA on the time spent escape-rearing revealed no significant between-subjects effects [housing treatment: F(1,13)=0.014, p=0.454) or within-subjects effects [trial: F(2,26)=0.275, p=0.762; minute: F(9,117)=0.850, p=0.572)].

Table 3.6 Number of rear bouts per trial. Data shown are means ± standard errors of the mean.

Group	N	TRIAL 1	TRIAL 2	TRIAL 3
control	7	5.3 ± 0.7	6.0 ± 1.3	5.9 ± 0.6
enriched	9	6.0 ± 1.5	6.7 ± 0.9	5.4 ± 0.9
yellow	8	6.5 ± 1.7	6.7 ± 1.0	6.5 ± 0.8
black	10	5.2 ± 0.9	6.2 ± 0.8	5.1 ± 0.6

Table 3.7 Time spent rearing per trial. Data shown are means ± standard errors of the mean.

Group	N	TRIAL 1 (s)	TRIAL 2 (s)	TRIAL 3 (s)
control	7	191.0 ± 59.9	121.0 ± 41.3	136.9 ± 21.3
enriched	9	69.1 ± 18.2	111.7 ± 21.7	133.8 ± 32.1
yellow	8	94.9 ± 21.1	88.1 ± 18.5	135.7 ± 21.1
black	10	138.9 ± 48.5	132.4 ± 31.3	134.9 ± 39.8

Individual differences in exploratory behavior:

There were individual differences in tongue flick counts over time (tongue flick counts per minute range: minimum = 1 and maximum = 102); selected differences are shown in Figure 3.10.

Part 4 Learning study

Latency to goal hole:

Descriptive statistics are presented in Table 3.8. A repeated measures ANOVA on latency in traveling to the goal hole (Table A.22, Figure 3.11A, B) revealed significant effects of both housing treatment [$F(1,11)=7.810$, $p=0.004$] and clutch [$F(1,11)= 12.328$, $p=0.001$]. There was a significant effect of trial within subjects [Figure 3.12A; $F(2,22)=7.627$, $p=0.003$] with the second trial having the shortest latency. A repeated measures test of cue type on the latency to the goal hole resulted in marginally significant differences [Figure 3.12B, $F(2,41)=2.595$, $p=0.087$] with the no cue trial having the longest latency to the goal hole.

Table 3.8 Latency to the goal hole by day. Data shown are means ± standard errors of the mean.

Group	N	Day 1 (s)	Day 2 (s)	Day 3 (s)	Day 4 (s)	Total (s)
control	7	492.4 ± 28.5	498.4 ± 61.2	488.7 ± 37.9	504.0 ± 33.3	495.9 ± 29.6
enriched	9	445.1 ± 28.5	440.4 ± 61.2	440.1 ± 37.9	397.5 ± 33.3	430.8 ± 29.6
yellow	8	479.4 ± 34.0	536.4 ± 73.2	570.3 ± 45.3	493 ± 39.8	519.8 ± 35.4
black	10	458.2 ± 21.5	402.4 ± 46.3	358.4 ± 28.6	408.4 ± 25.2	406.8 ± 22.4

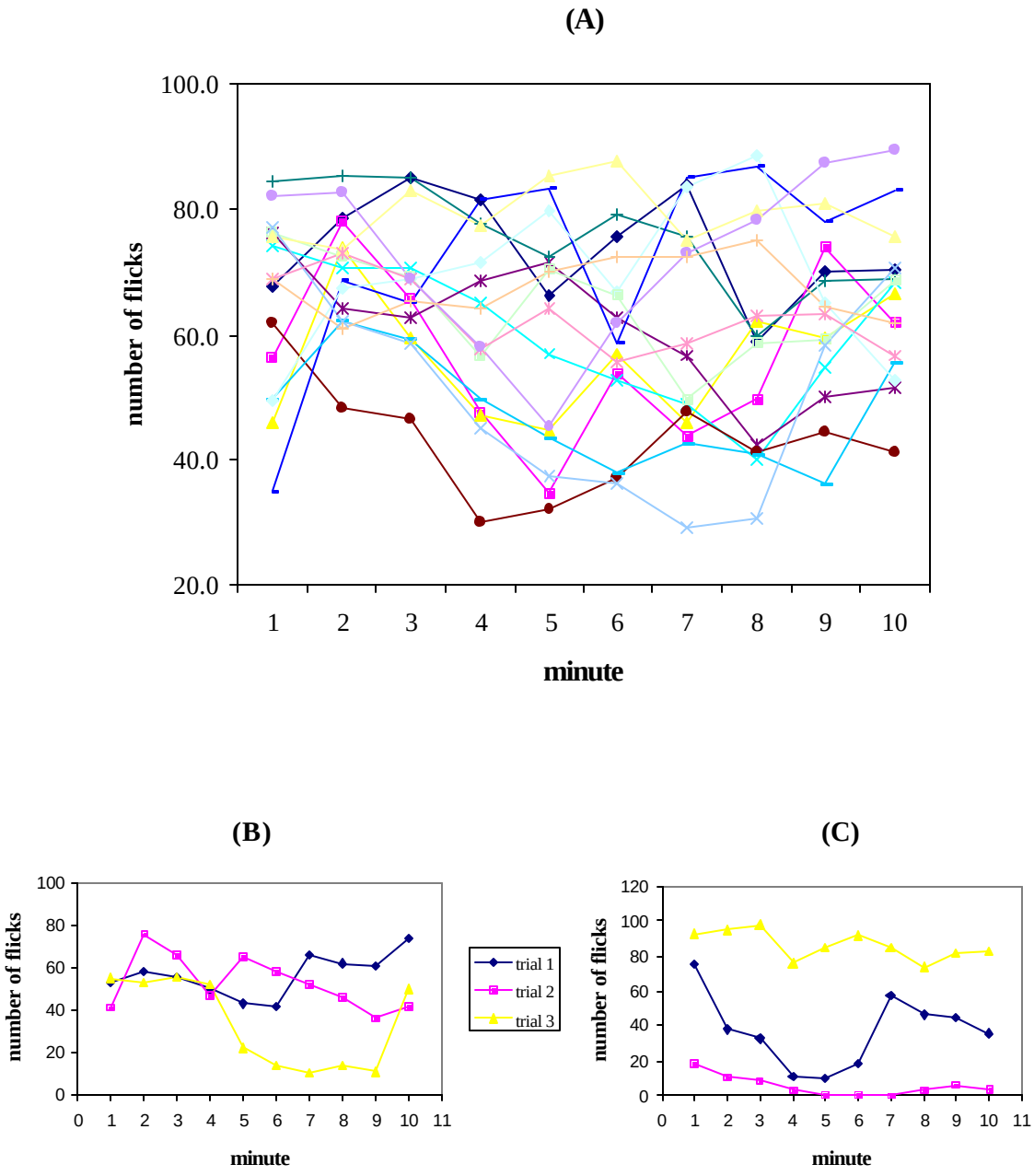

Figure 3.10: Individual variation in tongue flick behavior in response to an open field. (A) All subjects averaged across trials, each color represents a different snake. Representative variation in individuals for each trial: (B) All trials of snake ID #3; (C) All trials of snake ID #27.

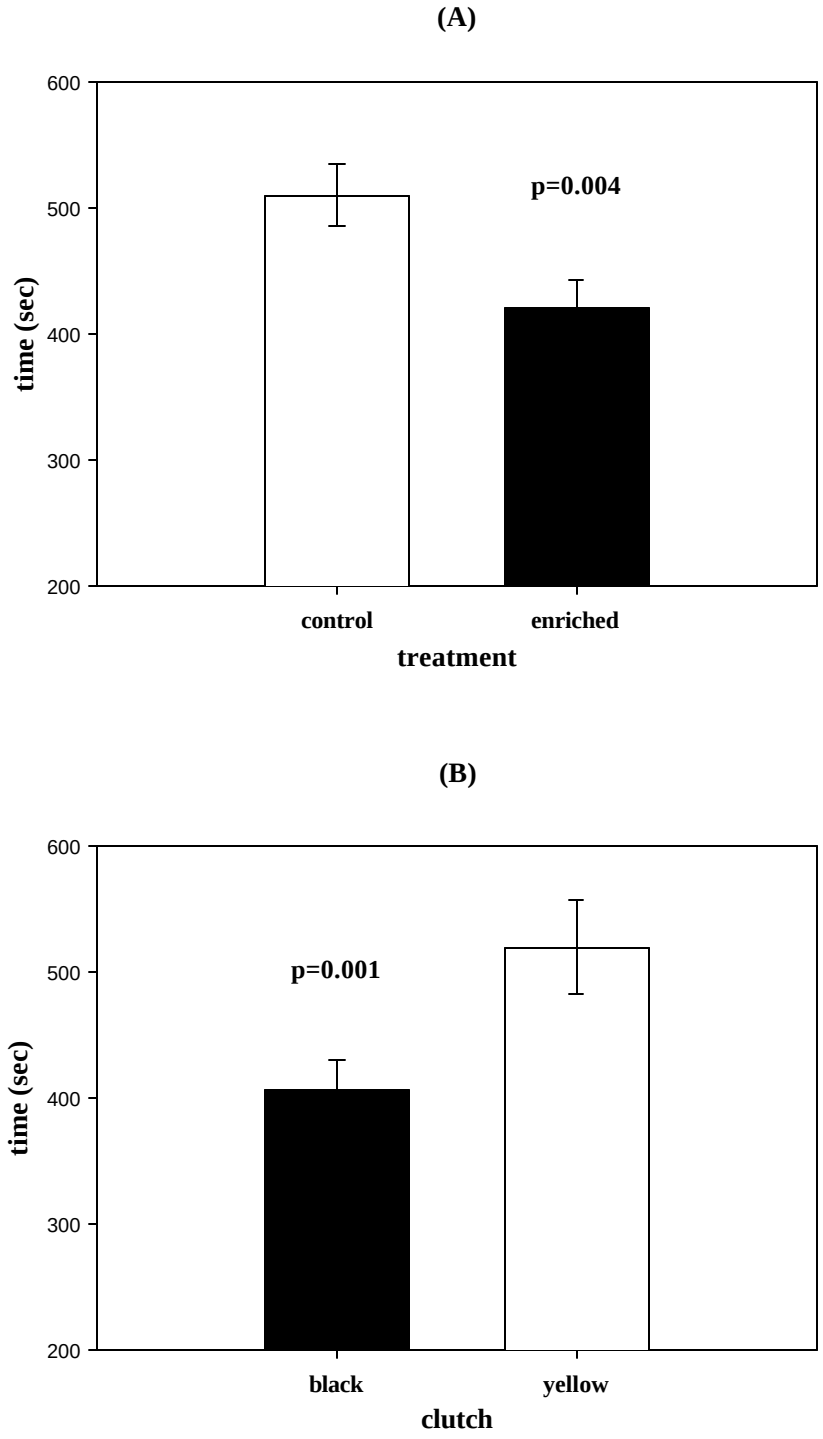

Figure 3.11: Effect of housing treatment and clutch on the latency to goal hole. (A) Latency to the goal hole by housing condition, (B) Latency to the goal hole by clutch. Shown are means +/- the standard error of the mean.

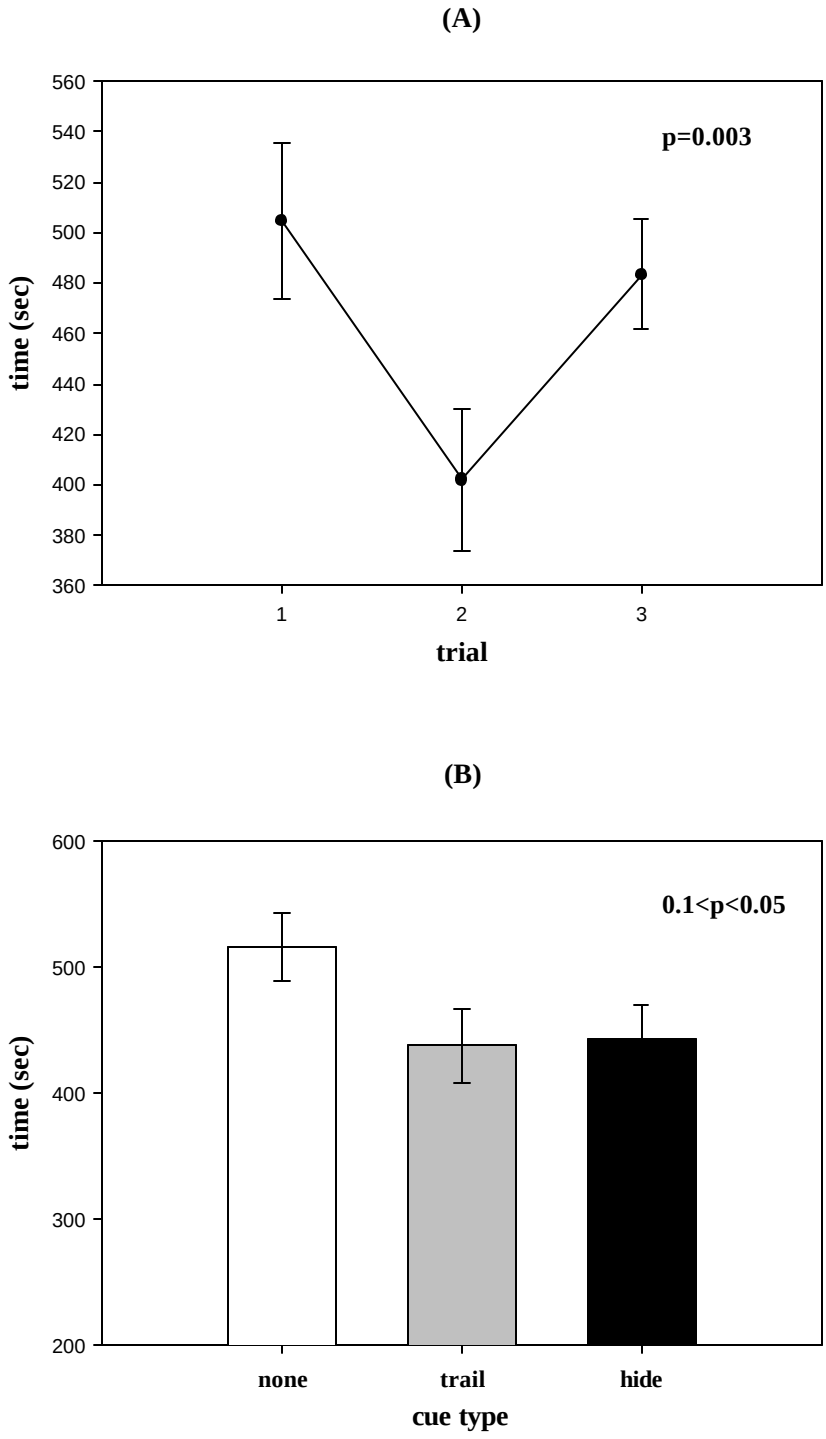

Figure 3.12: Effect of trial and cue type on the latency to the goal hole. (A) The latency to the goal hole by trial, (B) The latency to the goal hole by cue type. Shown are means +/- the standard error of the mean.

Table 3.9 Number of errors by day. Data shown are means ± standard errors of the mean.

Group	N	Day 1	Day 2	Day 3	Day 4	Total
control	7	1.7 ± 0.3	1.8 ± 0.5	1.9 ± 0.4	1.7 ± 0.3	1.8 ± 0.2
enriched	9	1.6 ± 0.3	2.2 ± 0.5	1.3 ± 0.4	1.5 ± 0.3	1.6 ± 0.2
yellow	8	1.9 ± 0.4	2.2 ± 0.6	1.8 ± 0.5	1.5 ± 0.3	1.6 ± 0.2
black	10	1.3 ± 0.3	1.7 ± 0.4	1.4 ± 0.3	1.7 ± 0.2	1.9 ± 0.3

Table 3.10 Latency to the goal quadrant by day. Data shown are means ± standard errors of the mean.

Group	N	Day 1 (s)	Day 2 (s)	Day 3 (s)	Day 4 (s)	Total (s)
control	7	119.6 ± 32.5	227.8 ± 39.7	187.5 ± 29.6	210 ± 34.9	186.4 ± 18.0
enriched	9	143.6 ± 28.9	164.8 ± 35.4	136.2 ± 26.3	206.1 ± 31.2	162.7 ± 16.1
yellow	8	137.3 ± 36.2	245.6 ± 44.2	184.9 ± 32.9	266.2 ± 38.9	208.5 ± 20.1
black	10	125.9 ± 26.0	146.9 ± 31.7	138.8 ± 23.7	150.5 ± 27.9	140.5 ± 14.4

Number of errors:

Descriptive statistics are presented in Table 3.9. A repeated measures ANOVA revealed no significant differences in the number of errors (i.e., non-goal hole pokes) either between-subjects [housing treatment: $F(1,11)=0.056$, $p=0.817$] or within-subjects [day: $F(3,33)=1.151$. $p=0.343$; trial: $F(2,22)=0.896$, $p=0.423$].

Latency to goal quadrant:

Descriptive statistics are presented in Table 3.10. A repeated measures ANOVA revealed a significant difference in the latency in traveling to the goal quadrant between clutches in that black snakes had shorter latencies [Table A.23, $F(1,11)=7.574$, $p=0.009$ sec]. There were no other significant differences in latency either between or within subjects.

Time spent in goal quadrant:

Descriptive statistics are presented in Table 3.11. A repeated measures ANOVA revealed no significant differences in the time spent in the goal quadrant either between-

Table 3.11 Time spent in the goal quadrant by day. Data shown are means ± standard errors of the mean.

Group	N	Day 1	Day 2	Day 3	Day 4	Total (s)
control	7	111.4 ± 21.2	90.5 ± 22.1	112.6 ± 20.3	78.7 ±13.1	98.3 ± 11.2
enriched	9	97.3 ± 18.9	94.5 ± 19.7	66.8 ± 18.1	61.3 ± 11.6	79.9 ± 9.9
yellow	8	84.0 ± 23.6	89.4 ± 24.6	115.6 ± 22.6	74.8 ± 14.5	91.0 ± 12.4
black	10	124.7 ± 16.9	95.5 ± 17.7	63.8 ± 16.2	65.1 ± 10.4	87.3 ± 8.9

subjects [housing treatment: $F(1,11)=0.249$, $p=0.314$] or within-subjects [day: $F(3,33)=0.509$. $p=0.679$; trial: $F(2,22)=1.321$, $p=0.287$].

Part 5 Dicriminant function analysis

I also performed discriminant function analyses (DFA) to identify the linear combination of variables that best distinguished snakes raised in enriched environments from controls. For the morphological variable, size was standardized by dividing mass by snout-vent length. For the behavioral variables, the measures were averaged across all trials and/or days to give one value for that particular behavior. There was an overall significant effect of the discriminant function analysis with most morphological and behavioral variables included in the matrix [?=0.005, $?^2=42.919$, df=10, p=0.000]. The absolute values of the coefficients listed in Table 3.12 represent the contributions of each variable in the DFA; thus feeding behaviors and the time spent in the goal quadrant in the learning task contributed most to the analysis. Furthermore, 100% of the original grouped cases were correctly classified by the DFA into EC and IC housing groups (Table 3.13). In addition, by leaving out the morphological data, the DFA was still highly significant [?=0.023, $?^2=32.110$, df=9, p=0.000].

Table 3.12 Standardized canonical discriminant function coefficients.

Dependent variable	partial contribution
mass/SVL	-3.475
latency to goal hole in learning task	2.274
time to goal quadrant in learning task	9.279
number of errors in learning task	-2.038
number of tongue flicks in exploratory task	3.670
number of grids crossed in exploratory task	-.121
time spent rearing in exploratory task	3.850
number of rear bouts in exploratory task	-6.557
total consumption time with live prey	19.035
total consumption time with dead prey	-20.237

Table 3.13 Classification results for the discriminant function analysis.

			Predicted Group Membership		
		Treatment	control	enriched	Total
Original	Count	control	7	0	7
		enriched	0	8	8
	%	control	100.0	0	100.0
		enriched	0	100.0	100.0

a 100.0% of original grouped cases correctly classified.

Chapter 4: DISCUSSION

Providing environmental enrichment for laboratory and zoo animals is beneficial for animal welfare practices in addition to studying mechanisms of behavioral plasticity. The lives of captive animals have been improved from the results of these studies in that enriched housing conditions have shown to reduce appetitive behaviors and promote reproduction, which is important for the maintenance of threatened species (reviewed by Shepherdson et al., 1998). This study attempted to address the consequences of plasticity in morphology and behavior from a psychological point of view, although it has implications for captive animals and re-release programs.

The behavioral data did not demonstrate clear differences in exploratory behavior or learning abilities between EC and IC snakes in all the ways I had initially expected. For example, the EC snakes did not exhibit significantly higher exploratory behavior on their first exposure to the open field apparatus; although, they did habituate over repeated exposures to the open field. Additionally, EC snakes did not improve with experience with repeated feeding trials or learning trials. This result suggests that snakes cannot be enriched in the same way as some previously studied animals (see discussion below). Furthermore, the housing paradigm and the behavioral tests that I used in this project may not have been appropriate to tease out more differences between the housing conditions. For example, the fact that the EC snakes remained in their housing conditions for eight months (compared to 30 days for rodent studies) could have abolished any positive effects of enrichment as the environment was no longer stimulating to them. In fact, rodent studies have compared different levels of stimulation in enriched environments and demonstrated that behavioral effects depend on the relative stimulus complexity of those environments (Zimmermann et al., 2001).

In addition to investigating treatment effects of differential housing on snakes, clutch differences were also studied. Because the sample size was very small (i.e., two), the significant clutch effects were not of primary importance to the study but should be mentioned. Ideally, I would have tested several different clutches to be able to look at genotype-environment interactions (Burghardt et al., 2000). In that way, I could have

isolated any environmental effects on behavior as the subjects would be genetically similar. However, in this study, the subjects were not only from two clutches, they were also subspecies (namely *Elaphe o. obsoleta* and *Elaphe. o. quadrivittata*). Although these subspecies have similar ecologies (Conant & Collins, 1991), the subtle differences in their natural history could manifest substantially in the behaviors studied in this experiment. For example, the yellow snakes seemed to be crepuscular whereas the black snakes seemed to be diurnal. However, clutch differences in the behavior of animals from the same population of the same subspecies are well-documented in snakes (Brodie & Garland, 1993) and so conclusions on the role of subspecies in the differences found here is premature.

Further examination with environmental enrichment in other species of snakes and with many more subjects is needed for conclusive results on environmentally induced plasticity in snakes. Nevertheless, the fact that the data presented here do show some effects of enrichment, in spite of the very small sample size using two clutches with very different genetic backgrounds suggests that more large scale and intensive studies will show even more significant effects (both biologically and statistically).

Part 1 Design of environmental enrichment

In developing the housing conditions classified as environmentally enriched in this study, I tried to apply critical anthropomorphism in my design (Rivas and Burghardt, 2001). In other words, I attempted to incorporate what Jacob von Uexküll termed Innenwelt and Umwelt, or the inner world of animals and how they perceive and respond to their environment, respectively (von Uexküll, 1909). This framework is extremely important when designing behavioral studies in animals quite different from ourselves. It is quite possible that my perception of what was stimulating to the snakes was actually quite mundane. Future experiments on environmental enrichment in snakes might consider providing odor stimuli and implementing an element of unpredictability such as varied feeding times and object introduction.

The results of each experiment are discussed below.

Part 2 Morphological study

Overall, body morphology was affected by both clutch and treatment group. Although all snakes were offered the same amount of food, they did not all consume the same amount. For example, EC snakes tended to eat more than the IC snakes. Not surprisingly, EC snakes attained larger body and head sizes most likely through increased growth rates; however, this change was not entirely related to the amount of food consumed. It is difficult to determine what caused this increased growth rate in the EC snakes above and beyond the amount of food consumed. Differing activity levels between the treatment groups could have contributed to this difference, although, I can only speculate on this possibility. My behavioral results did not show significant differences in activity levels (e.g., the number of grids crossed in the exploratory studies) but daily activity levels (leading to changes in muscle mass perhaps) may have differed between the housing treatment groups. For example, I tended to observe the EC snakes climbing on the vertical branches in their cages, whereas the IC snakes were typically found in their hide boxes. The lack of systematic data on the activities of these animals over the eight month enrichment period prevents forming a possible explanation of whether this increased growth rate seen with the EC snakes was due to differential activity.

It was not unreasonable to predict changes in bone morphology in an eight month treatment period as all snakes underwent substantial growth in both mass and snout-vent length during that time. My results were similar to those reported for wild caught animals in that yearlings ranged in lengths from 500-600 mm (Stickel et al., 1980; Fitch, 1963). Furthermore, growth rates for the EC snakes (27.5 mm/month compared to 22 mm/month for IC snakes) approached those of wild caught snakes at averages of 30 mm/month (Fitch, 1963). It would be more likely to observe morphological plasticity during development and, in fact, most studies have been conducted in neonates (e.g., Queral-Regil & King, 1998; Bonnet et al., 2001). However, Krause et al. (in press) and Forsman (1991) did find differences in natural populations of adult snakes feeding on different prey items. In addition, Forsman's study (1996) also found morphological

differences between body size but not head size in only 14 weeks in a captive population of *Vipera berus*.

Since both feeding frequency and prey size were kept constant in this study, the mechanical strain on the muscles to constrict and maneuver live prey may not be significant enough to result in changes in bone morphology. Although previous studies in fish (Wimberger 1992, 1993) have demonstrated that the way in which they process food can lead to differently shaped bone via differing mechanical strain (and partly via nutritional differences), the fish were fed different diets from hatching. I am unaware of a similar study on morphological plasticity due to differential kinematics of feeding in adult animals.

Part 3 Behavioral studies

Feeding study:

Environmental enrichment studies in general include object introduction/ manipulation as part of the enrichment procedure. In this study, a feeding component was included because snakes, being limbless, cannot manipulate objects except during prey handling. Since IC snakes did not receive live prey, they did not have any opportunity in their home cage for either object manipulation or stimulation. Although necessary, this situation also complicates the results of my study: because the snakes did not receive identical feeding experience, any improvements in feeding efficiency may be due to experience as opposed to the physical aspects of the environmental enrichment. It is known that rat snakes require feeding experience to develop efficient prey handling skills (Greene, 1977; Mori, 1996, Mehta, in press); thus, if the IC snakes had the opportunity to manipulate live prey in their standard environments, perhaps they would have performed similarly to EC snakes.

It is interesting to note that, unlike Mori's study (1996), none of the categorical data analyses revealed significant differences in treatment groups, however some trends were observed. Furthermore, differences in handling time (be it successful or unsuccessful) may be due to trends that were seen in the capture and ingestion behaviors.

For example, only IC snakes swallowed dead prey tail first (which typically leads to longer ingestion times, personal observation).

For this study, I classified feeding proficiency behaviors as decreased missed capture attempts, decreased unsuccessful handling time, and decreased handling time. On average, the EC snakes had decreased consumption times, but also had increased unsuccessful feeding attempts. Although only one of the criteria for feeding proficiency was met, the significantly decreased latency in consumption time (handling plus swallowing time) in EC snakes could possibly classify them as more efficient feeders. However, the observed change in feeding efficiency may be related to size differences between the treatment groups. Unlike Krause & Burghardt's study (2001) in *Thamnophis sirtalis,* in which there were no differences in head sizes between the treatment groups, the larger head sizes in the EC snakes may have allowed for increased feeding efficiency. Separating morphological plasticity from feeding behavior may be particularly important in such gape-limited predators (see study in sticklebacks by Day & MacPhail, 1996).

Exploratory study:

From the rodent enrichment studies, I had predicted that EC snakes would initially increase their exploratory behavior and then have a marked habituation. In rodents, enrichment reduced the latency to explore the open field (Zimmermann et al, 2001); however, there was no obvious effect of treatment on any of the exploratory behaviors and thus I cannot conclude that EC snakes had increased exploratory behavior as expected. However, my hypothesis that EC snakes would habituate (to repeated exposures to the open field apparatus) more quickly than controls was correct. EC snakes had a significant decrease in tongue flick rates and ambulation across trials as compared to IC snakes (in both cases, IC snakes had increasing trends across trials). Zimmermann et. al. (2001) report that most differences between EC and IC rats can be explained in terms of differences in within-session habituation: no initial differences in exploratory behavior, but significant differences toward the end of the session. In contrast, my experiment with snakes showed the opposite trend in that differences were seen mainly in habituation across trials as opposed to within-trials.

The behavior actually being measured in an open field task has been the subject of much debate. Renner & Rosenzweig (1987) have described a subject's behavior in the open field as an attempt to get out of the apparatus, not exploration of a novel environment. Since the open field test was initially designed to study emotionality in rats, it is understandable that there may be a conflict between the animal's tendency to explore and high degrees of anxiety from being in a closed space. Because I tested these snakes in the dark, I had hoped to isolate this potential exploratory behavior over escape behavior. However, the behavior that I termed "escape-rearing" seemed to function for just that – escape. All snakes tended to engage in thigmotaxis with intermittent bouts of rearing, especially when they reached a corner of the apparatus. I did see a trend in EC snakes in that they seemed to have more rearing bouts, but spent less time rearing per bout than IC snakes. I can only speculate that perhaps the EC snakes were exploring more of the apparatus to find an escape route or, alternatively, learned that spending a long time rearing per bout did not increase their chances of escape.

The open field task may not have been ecologically relevant for snakes in this type of study. Furthermore, the measurements of tongue flicking and grid crossing may not have been appropriate to reliably predict exploratory behavior. Greenberg (1993) noted that tongue flicking occurs in many contexts in addition to exploration and may be caused by a variety of factors including behavioral arousal and stress. Although these factors are important in studying environmental influences on behavior, the task was not designed to tease apart these variables. Future studies should investigate these perhaps more important indicators of normal behavior.

Learning study:

Learning ability may be the most important measure of environmental enrichment effects on behavior, as the brain region typically affected by enrichment is the cortex. The test arena I used was designed after a previously published learning study in snakes (Holtzman et al., 1999). My personal experience with training snakes has been wrought with inconsistencies. As per Holtzman et al., (1999), the motivation for the snake to find the goal hole was to escape the heated test arena to retreat to a dark moist refuge. In a

preliminary study with rat snakes, the snakes could not find the goal hole under those conditions, thus I added odor cues to the apparatus. In my experiment, there were no significant treatment by trial or day interactions, which demonstrated that there were no differences in the learning ability of the EC or IC snakes over time. This result may have been due to the few trials that I conducted as compared to other learning and maze studies, which may include over two weeks of training. However, the significant treatment effect for latency to the goal hole may explain an overall ability for the EC snakes to learn the task. Although I used to odor cues to provide a more ecological relevant stimuli, the addition of odor to the task may have either confused the snakes or may have not been a strong enough motivator for them to find the goal hole quickly and consistently. However, all groups did show a trend in finding the goal hole faster when they were exposed to an odor trial or odor in the goal hole as compared to no odor cue at all.

Previous enrichment studies have only reported differences in EC and IC animals in complex tasks. Relatively simple tasks do not seem to yield consistent results; furthermore, IC animals tend to match their counterparts when given enough training (cited in Renner & Rosenzweig, 1987). The learning task in this study may not have been appropriate for snakes or at least not appropriate to reveal differences in housing conditions. Since the EC snakes were provided with branches for vertical locomotion and substrate to burrow in, a more relevant task of learning may have utilized these differences in habitat complexity. Furthermore, because chemosensation is so important to snake behavior, a learning task focusing directly on odor cues may be more ecologically relevant.

The benefits of environmental enrichment may be difficult to test in standard learning paradigms, which may affect all experimental subjects and not just reptiles. Although environmental enrichment is supposed to induce its effects through latent learning and thus differences should be apparent in most learning tasks, actually determining an appropriate task to observe these differences may be challenging. As suggested by Renner & Rosenzweig (1987), testing a behavior such as predator avoidance may be a more relevant task for the benefits of enrichment, yet most

enrichment treatments do not allow for experience with predators (for predatory experience in tamarins, see Moodie & Chamove, 1990). It is interesting to note that many studies of environmental enrichment do not consider the type of enrichment when developing the tasks to measure behavioral change.

Part 4 Discriminant function analysis

Discriminant function analysis (DFA) revealed that on the basis of my experimental variables, the subjects used in this study can be assigned to their appropriate treatment groups. This is an exciting result because on the basis of the ANOVAs for each individual experiment, it seemed as if the housing treatment groups did not differ enough to show an overall effect of enriched rearing. Furthermore, when looking only at the behavioral data (which is more similar to the typical enrichment studies), the DFA also revealed significant results and classifies each individual in the appropriate group.

Part 5 Implications of the study

One of the most important questions in biology is how the brain changes both structurally and functionally throughout the lifetime of an individual. It is clear that experience induces behavioral change and this phenomenon has been examined in species as diverse as *Drosophila* and humans. However, there is a gap in the literature concerning the range of species for which the effects of environmental complexity have been studied. This research project will be the first study to systematically investigate environmental influences due to housing condition on the behavior of reptiles. Reptiles have frequently been neglected (Chiszar et al., 1976; Burghardt, 1977; Burghardt, 1996) in studies involving the brain and learning behavior (except for chemosensation) because of the difficulty in providing the appropriate conditions and motivation (Glickman & Sroges, 1966). Despite this experimental design problem, their tendency to be non-social (thus eliminating the confounding effects of social and non-social stimulation), and their extreme dependence on their environment, make reptiles an excellent study animal for studies on the environmental impacts on behavior. Furthermore, large reptiles demonstrate unexpected complexity in cognitive abilities such as the foraging strategies

of the white throated monitor (*Varanus albigularis*; Kaufman et al., 1996) and the investigative behavior of a captive Komodo dragon (*V. komodoensis*; Burghardt et al., 2002); thus, they may be quite amenable to environmental enrichment.

The results of this study are also relevant to captive husbandry practices and are crucial for the validity of laboratory-based behavioral research. It is interesting to note that two of my control snakes died during a stressful part of this project (moving their cages into another building for behavioral testing). Although inconclusive autopsies were conducted, it seems as if both snakes suffered from neurological problems (personal observation of D. O'Rourke, University of Tennessee). Although stereotypic behaviors are not prevalent in reptiles, the example of self-mutilation in a captive turtle (Burghardt et al., 1996) may imply that they are possible and can be prevented with environmental enrichment.

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APPENDICES

Appendix 1: EXPERIMENTAL DEFINITIONS

The following pages contain definitions for the behaviors recorded in this project on sub-adult *Elaphe obsoleta.* The terminology used in this project is to similar to that used by others (Mori, 1996; Krause & Burghardt, 2001; Mehta, in press).

Morphometry:

1. Head length: from the base of the skull to the tip of the snout
2. Cranial width: across the top of the head above the eyes
3. Jaw length: from the quadrato-articular joint to the tip of the snout
4. Eye width: diameter of the lens
5. Interocular length: from the inside corner of each eye
6. Snout-vent length: from the tip of the snout to the anterior portion of the vent
7. Tail length: from the posterior portion of the vent to the tip of the tail
8. Mass: the amount the snake weighs

Feeding study:

1. Condition of prey: live or dead
2. Capture position: body region seized by snake; anterior (head and shoulder), middle (trunk and forelimbs), posterior (pelvis, hindlimbs, and tail)
3. Prey-handling method: simple seizing (grasping prey in jaws and beginning ingestion), pinion (pressing the prey against the substrate by the snake's body; for simplicity, I also used this category to include "hair-pin loop," a term used to describe the behavior of squeezing the prey between non-overlapping portions of the snake's body, see Mori, Mehta), or constriction (coiling around prey)
5. Type of coil: regular (at least 2 full coils encircling the prey's body) and irregular (unstable coiling, widely spaced coils)
4. Prey position at ingestion: body region first ingested by snake; anterior (head and shoulder), middle (trunk and forelimbs), posterior (pelvis, hindlimbs, and tail)

5. Feeding proficiency: misses (number of missed attempts at subduing prey), unsuccessful handling time (time from initial prey strike that does not terminate in swallowing behavior), handling time (time from successful prey strike to when the prey is maneuvered into place so that it can be swallowed), and ingestion time (time from when snake begins side to side jaw movements until first post ingestion tongue flick)
6. Total feeding duration: time from prey strike to completion of prey ingestion.

Exploratory study:

1. Tongue flicks: extensions of the tongue. Each tongue flick will be scored.
2. Ambulation: movement by the snake in a horizontal plane. Counts will be made of grid squares crossed by the tip of the rostrum.
3. Escape-rearing: movement by the snake in a vertical plane. Several measures will be used: latency to rear, duration of rearing, and number of rearing bouts in one trial.

Learning study:

1. Latency: time until the snake has all of its body in the goal hide box and time until the tip of the rostrum crossed the goal quadrant boundary for the first time
2. Errors: non-goal hole pokes by the tip of the snake's head
3. Time spent in goal quadrant: amount of time from which the tip of the rostrum crossed the goal quadrant boundary until the goal was found or the tip of the rostrum crossed into another quadrant

Appendix 2: TABLES

Table A.1: Subject information taken before and after the experimental procedure (Y=yellow, *E. o. quadrivittata*; B=black, *E. o. obsoleta*).

ID#	clutch	sex	enriched?	when?	mass (g)	SVL (mm)	tail length (mm)	number of meals	grams consumed
1	Y	M	yes	before	69.25	530	130		
				after	125.51	778	170	33	231
2	Y	M	no	before	43.60	510	110		
				after	87.06	680	135	29	203
3	Y	F	yes	before	46.93	480	120		
				after	88.43	698	155	31	217
4	Y	M	yes	before	53.46	530	120		
				after	110.32	773	185	33	231
6	Y	M	no	before	47.19	500	120		
				after	78.44	667	160	27	189
7	Y	F	no	before	58.40	530	130	DIED	
10	Y	F	no	before	53.62	520	105	DIED	
12	Y	F	yes	before	50.06	500	95		
				after	95.14	647	90	30	210
19	B	M	yes	before	58.10	530	100		
				after	144.25	725	148	30	210
20	B	M	no	before	61.03	520	100		
				after	104.71	665	119	27	189
21	B	M	yes	before	61.28	530	110		
				after	133.55	747	149	29	203
22	B	F	no	before	63.84	520	105		
				after	98.43	690	129	26	182
23	B	F	yes	before	70.21	540	115		
				after	175.60	795	167	35	245
24	B	M	no	before	83.04	550	115		
				after	120.55	717	134	27	189
25	B	F	yes	before	74.70	530	100		
				after	140.15	760	163	29	203
26	B	M	yes	before	52.10	510	95		
				after	125.80	745	140	33	231
27	B	M	no	before	42.90	480	95		
				after	102.30	678	130	32	224
29	B	F	no	before	71.82	530	105		
				after	142.10	737	143	34	238

Table A.2 Subject information on head dimensions after experimental procedure (Y=yellow, *E. o. quadrivittata*; B=black, *E. o. obsoleta*).

ID #	clutch	enriched?	head length (mm)	head width (mm)	jaw length (mm)	interocular dist. (mm)	eye diam. (mm)
1	Y	Yes	18.30	13.98	19.20	9.46	3.43
2	Y	No	17.29	13.40	18.10	9.46	3.82
3	Y	Yes	17.82	14.18	19.49	9.36	3.42
4	Y	Yes	19.20	14.68	18.25	10.86	3.97
6	Y	No	17.87	14.22	18.51	10.08	3.67
12	Y	yes	16.34	13.28	17.35	9.33	3.58
19	B	Yes	19.08	14.65	20.45	9.53	4.03
20	B	No	18.04	12.32	20.19	9.46	4.04
21	B	Yes	19.40	12.53	20.09	9.39	3.83
22	B	No	17.69	12.43	19.11	9.07	4.05
23	B	Yes	19.26	14.17	20.75	9.69	3.75
24	B	No	19.04	13.85	20.55	9.31	3.96
25	B	yes	19.20	12.19	20.81	10.28	4.06
26	B	Yes	18.59	13.07	20.54	8.88	3.87
27	B	No	17.60	13.16	18.94	9.24	3.97
29	B	No	18.33	14.25	20.44	10.24	3.58

Table A.3 MANCOVA results for treatment and clutch differences in body mass and snout-vent length (SVL).

Effect	Wilks' Lamdba	F	Hypothesis df	Error df	Sig.
COVARIATE (MEALS)	.236	7.274	4.000	9.000	**p=0.007**
CLUTCH	.219	8.038	4.000	9.000	**p=0.005**
TREATMENT	.441	2.851	4.000	9.000	**p=0.044**

Table A.4 Univariate results for treatment and clutch differences in body mass and snout-vent length (SVL).

Source	Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
CLUTCH	MASS	4554.800	1	4554.800	22.434	**p=0.000**
	MASS GROWTH	1885.237	1	1885.237	20.609	**p=0.001**
	SVL	2664.396	1	2664.396	2.362	p=0.150
	SVL GROWTH	375.883	1	375.883	.766	p=0.399
TREATMENT	MASS	946.621	1	946.621	4.662	**p=0.025**
	MASS GROWTH	645.023	1	645.023	7.051	**p=0.011**
	SVL	3871.875	1	3871.875	3.433	**p=0.045**
	SVL GROWTH	2362.515	1	2362.515	4.815	**p=0.025**
MEALS	MASS	1230.393	1	1230.393	6.060	**p=0.030**
	MASS GROWTH	1391.913	1	1391.913	15.216	**p=0.002**
	SVL	4671.529	1	4671.529	4.141	p=0.065
	SVL GROWTH	5149.800	1	5149.800	10.496	**p=0.007**
ERROR	MASS	2436.409	12	203.034		
	MASS GROWTH	1097.709	12	91.476		
	SVL	13535.976	12	1127.998		
	SVL GROWTH	5887.539	12	490.628		

Table A.5 MANCOVA results for tests of treatment and clutch effects on head dimensions. Head length (HL), jaw length (JL), interocular distance (OD), and eye diameter (ED).

Source of variation	Wilks'Lambda	F	Hypothesis df	Error df	Sig.
COVARIATE (SVL)	.330	2.842	5.000	7.000	P=0.071
TREATMENT	.890	.174	5.000	7.000	p=0.471
CLUTCH	.174	6.660	5.000	7.000	**P=0.006**

Table A.6 Univariate results for HL, HW, JL, OD, and ED differences between clutches.

Source	Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
SVL	HL	3.714	1	3.714	15.774	**p=0.002**
	HW	1.475	1	1.475	2.448	p=0.144
	JL	1.292	1	1.292	3.497	p=0.086
	OD	.945	1	.945	4.105	p=0.066
	ED	.006	1	.006	.152	p=0.703
CLUTCH	HL	.935	1	.935	3.970	**p=0.070**
	HW	2.675	1	2.675	4.440	**p=0.057**
	JL	7.654	1	7.654	20.724	**p=0.001**
	OD	.617	1	.617	2.681	p=0.127
	ED	.233	1	.233	6.326	**p=0.027**
TREATMENT	HL	.003	1	.003	.012	p=0.457
	HW	.268	1	.268	.445	p=0.259
	JL	.014	1	.014	.038	p=0.425
	OD	.274	1	.274	1.192	p=0.148
	ED	.002	1	.002	.050	p=0.414
ERROR	HL	2.573	11	.234		
	HW	7.207	11	.655		
	JL	4.319	11	.393		
	OD	2.758	11	.251		
	ED	.425	11	.039		

Table A.7 Summary of categorical data for the feeding behaviors. Numbers presented as percents of total feeding episodes.

Type	Behavior	Type	Enriched	Control	Yellow	Black
TOTAL PREY	prey capture position	head	70.6	60.7	54.2	73.7
		middle	20.6	21.4	25.0	18.4
		tail	8.8	17.9	20.8	7.9
	handling method	coil	55.9	50.0	58.3	50.0
		pinion	11.8	10.7	12.5	10.5
		seize	32.4	39.3	29.2	39.5
	prey ingestion position	head	88.2	82.1	91.7	81.6
		middle	0.0	0.0	0.0	0.0
		tail	11.8	17.9	8.3	18.4
DEAD PREY	prey capture position	head	75.0	50.0	58.3	66.7
		middle	25.0	28.6	25.0	27.8
		tail	0.0	21.4	16.7	5.6
	handling method	coil	18.8	14.3	25.0	11.1
		pinion	12.5	7.1	16.7	5.6
		seize	68.8	78.6	58.3	83.3
	prey ingestion position	head	87.5	85.7	91.7	83.3
		middle	0.0	0.0	0.0	0.0
		tail	12.5	14.3	8.3	16.7
LIVE PREY	prey capture position	head	66.7	71.4	50.0	80.0
		middle	16.7	14.3	25.0	10.0
		tail	16.7	14.3	25.0	10.0
	handling method	coil	88.9	85.7	91.7	85.0
		pinion	11.1	14.3	8.3	15.0
		seize	0.0	0.0	0.0	0.0
	prey ingestion position	head	88.9	78.6	91.7	80.0
		middle	0.0	0.0	0.0	0.0
		tail	11.1	21.4	8.3	20.0

Table A.8 Pearson chi square analysis for prey capture position.

Source	Prey type	trial	df	Chi square	Significance
TREATMENT	DEAD	1	2	3.229	p=0.179
		2	2	4.148	p=0.123
	LIVE	1	2	1.778	p=0.206
		2	2	0.830	p=0.330
CLUTCH	DEAD	1	2	1.371	p=0.712
		2	2	4.622	p=0.202
	LIVE	1	2	0.356	p=0.837
		2	2	3.484	p=0.175

Table A.9 Pearson chi square analysis for prey ingestion position.

Source	Prey type	trial	df	Chi square	Significance
TREATMENT	DEAD	1	2	0.840	p=0.329
		2	2	0.840	p=0.329
	LIVE	1	2	0.085	p=0.386
		2	2	1.371	p=0.121
CLUTCH	DEAD	1	2	2.215	p=0.330
		2	2	0.738	p=0.769
	LIVE	1	2	0.356	p=0.551
		2	2	0.640	p=0.424

Table A.10 Pearson chi square analysis for prey handling method.

Source	Prey type	trial	df	Chi square	Significance
TREATMENT	DEAD	1	2	2.872	p=0.206
		2	2	0.875	p=0.416
	LIVE	1	2	0.788	p=0.369
		2	2	0.830	p=0.181
CLUTCH	DEAD	1	2	4.185	p=0.242
		2	2	1.067	p=0.785
	LIVE	1	2	0.027	p=0.869
		2	2	0.640	p=0.424

Table A.11 Repeated measures ANOVA results for missed attempts both between and within subjects.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
CLUTCH	1.337	1	1.337	.261	p=0.614
TREATMENT	5.968	1	5.968	1.164	p=0.145
TYPE	27.286	1	27.286	5.323	**p=0.029**
ERROR	138.396	27	5.126		
TRIAL	1.124	1	1.124	.211	p=0.649
TRIAL * CLUTCH	1.480	1	1.480	.278	p=0.602
TRIAL * TREATMNT	.258	1	.258	.048	p=0.414
TRIAL * TYPE	7.273	1	7.273	1.368	p=0.252
ERROR (TRIAL)	143.576	27	5.318		

Table A.12 Repeated measures ANOVA results for unsuccessful handling time both between and within subjects.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
CLUTCH	2800.692	1	2800.692	.211	p=0.649
TREATMENT	4395.771	1	4395.771	.332	p=0.285
TYPE	80745.434	1	80745.434	6.090	**p=0.020**
ERROR	357994.809	27	13259.067		
TRIAL	2511.008	1	2511.008	.145	p=0.706
TRIAL * CLUTCH	3617.681	1	3617.681	.209	p=0.651
TRIAL * TREATMNT	1172.724	1	1172.724	.068	p=0.399
TRIAL * TYPE	44027.020	1	44027.020	2.543	p=0.122
ERROR (TRIAL)	467366.223	27	17309.860		

Table A.13 Repeated measures ANOVA results for handling time both between and within subjects.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TYPE	124942.682	1	124942.682	16.529	**p=0.000**
CLUTCH	18608.031	1	18608.031	2.462	p=0.128
TREATMENT	8557.334	1	8557.334	1.132	p=0.149
ERROR	204095.970	27	7559.110		
TRIAL	42294.689	1	42294.689	6.423	**p=0.017**
TRIAL * TYPE	.857	1	.857	.000	p=0.991
TRIAL * CLUTCH	959.312	1	959.312	.146	p=0.706
TRIAL * TREATMNT	10847.379	1	10847.379	1.647	p=0.105
ERROR (TRIAL)	177783.781	27	6584.584		

Table A.14 Repeated measures ANOVA results for swallowing time both between and within subjects.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
CLUTCH	108403.982	1	108403.982	17.057	**P=0.000**
TREATMENT	3017.527	1	3017.527	.475	p=0.249
TYPE	8551.346	1	8551.346	1.346	p=0.256
ERROR	171598.289	27	6355.492		
TRIAL	87.540	1	87.540	.018	p=0.895
TRIAL * CLUTCH	5362.628	1	5362.628	1.089	p=0.306
TRIAL * TREATMENT	1054.434	1	1054.434	.214	p=0.324
TRIAL * TYPE	629.601	1	629.601	.128	p=0.723
ERROR (TRIAL)	132979.340	27	4925.161		

Table A.15 Repeated measures ANOVA results for consumption time both between and within subjects.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TREATMENT	103832.135	1	103832.135	5.059	**p=0.017**
CLUTCH	332129.386	1	332129.386	16.182	**p=0.000**
TYPE	45786.353	1	45786.353	2.231	p=0.147
ERROR	554169.795	27	20524.807		
TRIAL	59531.962	1	59531.962	8.062	**p=0.008**
TRIAL * TREATMNT	14515.568	1	14515.568	1.966	p=0.086
TRIAL * CLUTCH	13690.170	1	13690.170	1.854	p=0.185
TRIAL * TYPE	474.688	1	474.688	.064	p=0.802
ERROR (TRIAL)	199363.164	27	7383.821		

Table A.16 Repeated measures ANOVA results for trial time both between and within subjects.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
CLUTCH	268952.741	1	268952.741	6.154	**p=0.020**
TREATMNT	6506.409	1	6506.409	.149	p=0.352
TYPE	297447.094	1	297447.094	6.806	**p=0.015**
ERROR	1180043.967	27	43705.332		
TRIAL	25140.728	1	25140.728	.813	p=0.375
TRIAL * CLUTCH	23745.522	1	23745.522	.768	p=0.389
TRIAL * TREATMENT	13715.488	1	13715.488	.443	p=0.256
TRIAL * TYPE	59275.161	1	59275.161	1.916	p=0.178
ERROR (TRIAL)	835147.571	27	30931.392		

Table A.17 Repeated measures ANOVA results on the effects of treatment condition and clutch on number of tongue flicks.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TREATMENT	2155.113	1	2155.113	.579	p=0.230
CLUTCH	27.827	1	27.827	.007	p=0.932
ERROR	48373.228	13	3721.018		
TRIAL	1360.538	2	680.269	.433	p=0.653
TRIAL * TREATMENT	10905.719	2	5452.860	3.469	**p=0.023**
TRIAL * CLUTCH	3297.540	2	1648.770	1.049	p=0.365
ERROR (TRIAL)	40873.050	26	1572.040		
MINUTE	5420.849	9	602.317	1.854	**p=0.066**
MINUTE * TREATMENT	3073.899	9	341.544	1.052	p=0.202
MINUTE * CLUTCH	3843.121	9	427.013	1.315	p=0.237
ERROR (MINUTE)	38002.882	117	324.811		

Table A.18 Repeated measures ANOVA results on the effects of treatment condition and clutch on the number of grid crossings.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
TREATMENT	53.131	1	53.131	1.631	p=0.112
CLUTCH	39.276	1	39.276	1.206	p=0.292
ERROR	423.477	13	32.575		
TRIAL	2.326	2	1.163	.096	p=0.909
TRIAL * TREATMNT	62.560	2	31.280	2.570	**p=0.048**
TRIAL * CLUTCH	12.460	2	6.230	.512	p=0.605
ERROR (TRIAL)	316.409	26	12.170		
MINUTE	90.385	9	10.043	2.234	**p=0.024**
MINUTE * TREATMNT	35.650	9	3.961	.881	p=0.272
MINUTE * CLUTCH	47.287	9	5.254	1.169	p=0.322
ERROR (MINUTE)	526.065	117	4.496		

Table A.19 Summary of the slope (m) and y-intercept (b) for the individual regressions on tongue flick counts for each 10 minute exploratory trial.

ID	enriched?	CLUTCH	m trial 1	m trial 2	m trial 3	b trial 1	b trial 2	b trial 3
1	yes	Y	7.21	3.4	-0.38	35.53	47.67	75.2
2	no	Y	-3.47	1.38	-0.64	90.4	66.2	79.93
3	yes	Y	1.74	-2.2	-4.14	46.93	65	56.47
4	yes	Y	-0.18	3.52	-0.88	68.47	53.67	72.53
6	no	Y	0.03	-0.06	-1.84	57.93	49.93	66.73
12	yes	Y	-3.28	0.17	-1.22	90.13	62.67	65.33
19	yes	B	0.42	1.3	-1.18	67.87	82.07	85.27
20	no	B	-0.84	-0.52	3.36	63.73	48.53	45.4
21	yes	B	-1.18	-1.02	-2.78	47	77.93	54
22	no	B	-2.44	-1.45	-2.88	67.33	73.6	76.93
23	yes	B	-2.24	-1.78	0.11	77.8	62.67	70
24	no	B	-1.64	-5.63	-1.31	74.73	80.67	73.8
25	yes	B	0.39	0.58	2.79	75.67	72.8	48.93
26	yes	B	-1.12	0.9	0.89	64.53	70.13	64.4
27	no	B	-0.53	-1.18	-1.62	39.93	12	95.2
29	no	B	-4.47	-0.73	-1.69	93.67	83.33	88.07

Table A.20 Summary of the slope (m) and y-intercept (b) for the individual regressions on the number of grid crossings for each 10 minute exploratory trial.

ID	enriched?	CLUTCH	trial 1	trial 2	trial 3	trial 1	trial 2	trial 3
1	yes	Y	0.42	0.04	0.05	-0.8	1.6	2.73
2	no	Y	-0.09	0.12	-0.18	2	1.73	3.67
3	yes	Y	0.02	-0.09	0	1.27	2.4	1.13
4	yes	Y	0.04	-0.01	0	2	2.07	1.93
6	no	Y	0.26	0.1	0.38	0.07	1.27	0
12	yes	Y	-0.78	-0.41	-0.19	8.87	5.27	3.07
19	yes	B	0.01	0.18	-0.3	3.13	2.8	3.27
20	no	B	-0.18	-0.27	0.24	2.2	4.2	0.6
21	yes	B	0.05	0.41	-0.16	0.53	0.07	1.2
22	no	B	-0.21	-0.21	-0.09	2.47	2.93	1.87
23	yes	B	-0.01	0.12	-0.04	2.87	1.73	2.53
24	no	B	-0.28	-0.69	-0.24	5.13	6.67	3.73
25	yes	B	-0.95	0	-0.39	13.3	6.33	4.53
26	yes	B	-0.16	0.23	-0.46	4.47	0.73	6.33
27	no	B	-0.53	0	-0.5	4	0	6.67
29	no	B	-0.88	-0.5	-0.22	7.53	5.33	3.93

Table A.21 Independent t-tests results for the difference scores between trials 1 and 3 for the number of tongue flicks and grids crossed.

Difference scores between trials 1-3	t-value	df	Sig. (1-tailed)	Mean Difference	Std. Error Difference
TONGUE FLICKS	1.847	14	.043	135.7937	73.53186
GRID CROSSINGS	1.850	14	.043	16.3175	8.81825

Table A.22 Repeated measures ANOVA results on the effects of treatment condition, clutch, and cue type on the latency to the goal hole.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
CLUTCH	562676.647	1	562676.647	12.328	**p=0.001**
TREATMENT	356460.835	1	356460.835	7.810	**p=0.004**
CUE	236888.996	2	118444.498	2.595	**p=0.087**
ERROR	1871395.437	41	45643.791		
DAY	7705.683	3	2568.561	.128	p=0.943
DAY * CLUTCH	166751.149	3	55583.716	2.761	**p=0.058**
DAY * TREATMENT	38157.639	3	12719.213	.632	p=0.300
ERROR (DAY)	664452.048	33	20134.911		
TRIAL	268135.901	2	134067.950	7.627	**p=0.003**
TRIAL * CLUTCH	4020.996	2	2010.498	.114	p=0.892
TRIAL * TREATMENT	46693.506	2	23346.753	1.328	p=0.143
ERROR (TRIAL)	386718.043	22	17578.093		

Table A.23 Repeated measures ANOVA results on the effects of treatment condition, clutch, and cue type on the latency to the goal quadrant.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
CLUTCH	189043.214	1	189043.214	7.574	**p=0.009**
TREATMENT	25200.295	1	25200.295	1.010	p=0.161
CUE	54486.113	2	27243.057	1.091	p=0.345
ERROR	1023346.141	41	24959.662		
DAY	203172.268	3	67724.089	2.332	p=0.092
DAY * CLUTCH	98955.667	3	32985.222	1.136	p=0.349
DAY * TREATMNT	60480.655	3	20160.218	.694	p=0.281
ERROR (DAY)	958262.414	33	29038.255		
TRIAL	41657.941	2	20828.970	.998	p=0.385
TRIAL * CLUTCH	16391.257	2	8195.629	.393	p=0.680
TRIAL * TREATMNT	33944.150	2	16972.075	.813	p=0.228
ERROR (TRIAL)	459104.259	22	20868.375		

VITA

Lynn Marie Almli was born February 13, 1974, in Athens, Ohio. Her parents, Robert and Sheila Almli currently reside in St. Louis with her brother, Todd Almli. Lynn received her B.A. from the University of California at Berkeley where she was on an athletic scholarship for swimming. She then worked in a Neurology laboratory at the University of California at San Francisco before coming to the University of Tennessee (UT). During her graduate career at UT in the Department of Ecology and Evolutionary Biology, she worked with Dr. Gordon Burghardt in the Reptile Ethology Laboratory. Lynn is currently pursuing her Ph.D. degree with Walter Wilczynski in the Institute for Neuroscience at the University of Texas at Austin.