Abstract

Case, Beth Catherine. Environmental Enrichment for Captive Eastern Box Turtles (*Terrapene carolina carolina*). (Under the direction of Dr. Phil Doerr).

The relatively recent rise in public concern for animal welfare has led to the improvement of the housing conditions for a wide variety of captive animals. Despite this heightened awareness, reptiles have been relatively ignored. There has been a long held misconception that reptiles are stoic, adaptable and highly tolerant to abnormal conditions. However, because reptiles have strong innate drives and are not subject to parental and social education, they may actually be less tolerant and less adaptable to an unnatural, captive environment.

This study examined the physiological and behavioral impact of housing conditions on the captive eastern box turtle (*Terrapene carolina carolina*), and determined if box turtles exhibit a preference for an enriched or barren environment. Thirty-eight box turtles were randomized to either a barren (flat newspaper substrate) or enriched (cypress mulch substrate, shredded paper and a hide box) enclosure for a period of one month. Complete blood counts, fecal corticosterone, and body weights were measured at the beginning and end of the treatment period. Behavior was also assessed during the study.

Turtles in enriched enclosures had a significantly lower heterophil to lymphocyte ratio (H/L) at the end of the treatment period, indicating they were less stressed than barren-housed turtles. Enriched-housed turtles also spent significantly less time engaged in escape behavior, suggesting they were more accepting of their housing conditions. There was no

significant difference in fecal corticosterone or body weight change between the two treatments.

Prior to treatment each turtle was placed in a preference test system in which it could move freely between a barren and enriched environment. Relative dwelling time was determined for each environment. Turtles showed a distinct preference for the enriched environment. After the one-month housing experiment turtles were reevaluated for preference to determine if previous housing experience affects choice. Turtles continued to prefer an enriched environment regardless of prior housing conditions.

This study shows that the captive housing environment can negatively or positively influence the physiology and behavior of box turtles. Housing modifications that encourage typical species specific behavior should be provided. For the box turtle these would include substrate in which to dig and items that permit hiding.

Environmental Enrichment for Captive Eastern Box Turtles (*Terrapene carolina carolina*)

by

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Dedication

Dedicated in loving memory of my Father, Walter B. Case (1925-1975), who had the foresight to move his family to the country and my Grandmother, Emilie Fieg Case (1894-1984), who taught me the joys of a walk through the woods.

Biography

I was born in Norristown, PA, the youngest of three girls. By age five I was living in the Pocono mountains in my father's hometown of Milford, PA. There, I grew up exploring the woods of our rural home. After graduation from Delaware Valley High School, I attended Davis and Elkins College in Elkins, West Virginia. While originally drawn there to play field hockey, I discovered the joys and tribulations of attending a small private college. Upon earning a Bachelor of Science in environmental science, I moved to Raleigh where the core of my family was now located and opportunity flourished.

After many fulfilling years working within the oncology service at the NCSU College of Veterinary Medicine, I decided to return to higher learning. After two "false starts" I finally found my vocation. I have been fortunate to be able to continue working at the College of Veterinary Medicine while pursuing a Masters degree in zoology. While this has extended the duration of obtaining my objective, it has been well worth it.

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Chapter 1. Introduction and Literature Review.

The animal welfare movement of recent decades has brought to light many ethical and moral issues regarding animals in captivity and has resulted in legislation such as the Animal Welfare Act of 1966 (Segal 1989). This heightened sensitivity towards the physical and psychological well-being of animals has advanced the relatively new husbandry technique of environmental enrichment (Sheperdson 1998). Environmental enrichment has been defined in many ways. In the context of this study, it is a technique to improve the physiological and psychological well-being of captive animals by providing appropriate environmental modifications (Newberry 1995).

Despite the increased concern for animal welfare and the rapid growth of environmental enrichment and its application in the husbandry of many animal species, reptiles have historically been ignored (Hayes et al. 1998). Even the Animal Welfare Act excludes reptiles and amphibians from its regulations (McCarthy 1992). There has been a long held misconception that reptiles are stoic, adaptable and highly tolerant of abnormal conditions. However, because reptiles have strong innate drives and are not subject to parental and social education, they may actually be less tolerant and less adaptable to an unnatural, captive environment (Warwick 1990, Warwick et al. 1995, Arena and Warwick 1995).

Housing for captive reptile and amphibian species is being addressed more frequently and carefully than in the past. Enclosure design has been evaluated for a number of species, including geckos (*Phelsuma guentheri*) (Wheler and Fa 1995), blue-tongued skinks (*Tiliqua* *sencoides*) (Kreger 1993a), Trionychid turtles (Warwick 1990), Nile soft-shelled turtles (*Trionyx triunguis*) (Burghart et al. 1996, Krause et al. 1999), and African clawed frogs (*Xenopus laevis*) (Kaplan 1993). In addition, general reptile husbandry guidelines have been established by a number of researchers (Pough 1991, Pough 1992, McCarthy 1992, Warwick et al 1995, Chizar et al. 1995). However, there is still a deficiency of quantitative research (Hayes et al. 1998, Gould 1998), and much of the current reptile husbandry and enrichment, if utilized, is derived from empirical information and not based on controlled experimentation.

Conversely, there are numerous publications in the literature on the use of enrichment for mammals. A variety of farm (Wood-Gush and Beilharz 1983, Fraser et al. 1991, Mench et al. 1998, Norgaard-Nielsen et al. 1993), laboratory (Hubrecht 1993, Lidfors 1997, Olsson and Dahlborn 2002) and zoo animals (Mellon et al. 1981, Quick 1984, Markowitz and La Forse 1987, Carlstead et al. 1991), especially the non-human primates (Pereira et al. 1989, Kessel et al. 1995, Brinkman 1996, Watson 1997, Cardinal and Kent 1998), have been studied extensively. While these studies provide a starting point for enrichment for animals in other taxa, they are not a substitute for such studies. In fact, these studies have proven the importance of considering the needs of the particular species (Wilson 1982, Perkins 1992, Roder and Timmermans 2002), and in some cases, the individual (Chamove and Anerson 1989, Line and Clarke 1989, Heinz et al. 1998), when designing enrichment programs.

The types of enrichment are innumerable, but can be divided into four main categories: social, sensory, feeding, and physical environment (Newberry 1995). Social enrichment is especially important in animals that commonly live within groups in the wild. Most primates are social creatures and are an excellent example of animals that should be provided with social companionship (Segal 1989, NRC 1998). There are many reports of improved well-being when primates are housed in appropriate social groups. Rhesus monkeys (*Macaca mulatta*) exhibit less abnormal behavior when housed in pairs or groups than those housed individually (Schapiro et al. 1996). The activity levels of both gorillas (*Gorilla gorilla*) (Wilson 1982) and orangutans (*Pongo pygnialous*) (Wilson 1982, Perkins 1992) correlates highly with the number of animals in their group. In addition, group living is important for acquiring certain skills, especially parental skills, which are learned by helping others in their social group (Cleveland and Snowdon 1984, Snowdon et al. 1985). However, social housing must be approached cautiously. Deleterious consequences, due to inappropriate grouping, have been reported in sloth bears (*Ursus ursinus*) (Meyer-Holzapfel 1968), ocelots (*Felis pardalis mitis*) (Da Silveira 1973) and clouded leopards (*Neofelis nebulosa*) (Croke 1997).

Food presentation is another common method used to enrich captive environments. In the wild foraging occupies a large portion, often greater than 50%, of the time budget of most species (Segal 1989, Croke 1997, NRC 1998). Traditional methods for feeding confined animals usually consist of infrequent meals of highly processed food, which require little time and effort to consume (Fiennes 1966). Simply scattering food items in straw substrate, hiding food, or freezing it in blocks of ice has resulted in increased foraging time, greater use of cage space, and decreased abnormal and aggressive behaviors in many different species (Chamove et al.1982, Baker 1997, Carlstead et al. 1991, Bayne et al. 1991, Forthmann et al. 1992, Shepherdson et al. 1993, Young et al. 1994, Murchison 1995, Pyle et al. 1996, Glick-Bauer 1997).

Sensory enrichment is probably the least considered enrichment technique although some examples are available. A pair of Lar gibbons (*Hylobates lar*) increased their activity and vocalization in response to the recorded songs of conspecifics (Shepherdson et al. 1989). Simulated sounds of a bird in flight resulted in increased general activity and decreased stereotypic behavior for a captive African leopard (*Panthera pardus*) (Markowitz et al. 1995). Different odors, lighting conditions and even televisions have been used in an attempt to reduce abnormal behavior in chimpanzees (*Pan troglodytes*) (Brent et al.1989, Ostrower and Brent 1997, Fritz et al. 1997).

Another logical starting point for an enrichment program is the physical space of the enclosure. Cage design and furnishings must facilitate an array of species-specific behaviors, such as locomotion, physical postures, play and exploration (Erwin et al. 1979, Periera et al. 1989, Segal 1989, Warwick 1995, NRC 1998, Roder and Timmermans 2002). Enrichment should be uniquely formulated for each species. Thorough understanding of the natural history and normal behavioral repertoire of the animal of interest is necessary to determine optimal enclosure design, construction material and placement of furnishings (Novak and Suomi 1988, Pough 1991, Pough 1992, Kreger 1993b, DeNardo 1998, Roder and Timmermans 2002).

Enrichment strategies should be objectively evaluated to determine their relative effectiveness. There are three basic approaches for assessing the benefits of enclosure enrichment. These include measuring physiological parameters, measuring behavioral changes and preference testing. Each has advantages and disadvantages, which have been debated extensively. Some believe physiological parameters are the optimal method to measure well-being because interpretation of behavior is ambiguous (Barnett and Hemsworth 1990, Lance 1992). Others argue that high variability and the impact of invasive collection techniques make physiologic measures unreliable (McDonald 1976, Novak and Drewsen 1989, van Rooijen 1990, Rushen 1991). Incorporating multiple methods would, perhaps, avoid the pitfalls of relying on any one method (Broom 1988, Lance 1992, Clark et al. 1997).

There are numerous physiological measures of stress and poor welfare. These include, anorexia, weight loss, heart rate changes, immunosuppression, changes in hormone production, and reproductive impairment (Morton and Griffiths 1985, Broom 1988, Yosef 1988, Miller-Schroeder and Paterson 1989, Rushen and de Passille 1992, Lance 1994, Guillette et al. 1995, Clark et al. 1997, Terlow et al. 1997). These indices differ in their capacity to detect acute versus chronic stress and their ease of evaluation. A commonly utilized physiological indicator of stress is glucocorticoid concentrations (Lance 1990, Pickering and Fryer 1994, Wingfield 1994). These stress hormones are released upon activation of the hypothalamic-pituitary-adrenal axis during stressful conditions (Selye 1976, Orchinik 1998). Glucorticoids can be measured directly in the plasma or in other bodily excretions, such as saliva and feces (Carlstead et al 1992, Vincent and Michell 1992, Harper and Austad 1999, Millspaugh et al. 2002). Plasma concentrations reflect a single point in time, and these levels can fluctuate drastically within minutes (Windle et al. 1998). Additionally, the restraint and pain associated with blood collection may confound results. In contrast, fecal sampling is non-invasive and reflects steroid production over an extended period of time (Harper and Austad 1999). In some situations, fecal glucocorticoid concentrations are a more appropriate alternative to plasma concentrations (Monfort et al. 1998, Terio et al. 1999, Wasser et al. 2000, Millspaugh et al. 2002). While glucocorticoids may be excellent indicators of acute stress, they may be less useful for monitoring chronic stress due to the negative feedback regulation of the system and suppression of glucocorticoid production (Keller-Wood and Dallman 1984, Walker 1994, Mar Sanchez et al. 1998, de Jong et al. 2000, Hinz and Hirschelmann 2000).

Chronic stress may be more accurately monitored by assessing immune function, as measured by changes in leukocyte count (Gross and Siegel 1983). Among the predictable cellular changes associated with stress-leukograms are an increase in heterophils (equivalent to mammalian neutrophils) and a decrease in lymphocytes. The heterophil to lymphocyte ratio (H/L) has proven to be a very reliable hematologic measure of stress in a variety of bird species (Gross and Siegel 1983, Maxwel land Robertson 1998, Puvadolpivod and Thaxton 2000a, Elston et al. 2000, Vleck et al. 2000, Parga et al. 2001, Scope et al. 2002). The H/L ratio has also been utilized as a stress indicator in reptile studies, including alligators (Morici et al.1997, Lance and Elsey 1999) and green turtles (Aguirre et al. 1995, Work et al. 1999, Work et al. 2001). This parameter is less variable and longer lasting than changes in serum or fecal glucocorticoids. Additionally, leukocyte changes manifest more slowly and therefore are less likely to be influenced by the process of collecting blood (Puvadolpivod and Thaxton 2000b).

Behavioral studies assess welfare based on the activities performed by the captive animal. Behavioral observation is non-invasive and provides insight to internal biology (Gillingham 1995). Behavioral indicators of poor welfare include difficulty in performing normal behavior, increased aggression, stereotypies, escape attempts, excessive licking, preening and self-mutilation (Meyer-Holzapfel 1968, Warwick 1987, Broom 1988). Knowledge of normal species-specific behavior in the wild is necessary, including understanding the natural reactions to adverse conditions, such as pain or predation.

Preference testing is a relatively simple, direct method of comparing alternative captive enclosures (van Rooijen 1983, Broom 1988, Fraser et al. 1993, Fraser and Matthews 1997). This approach of simply allowing an individual to choose between two or more housing conditions has been used extensively in farm and laboratory animals (Hughes 1976, Dawkins 1976, Dawkins 1977, Arnold and Estep 1994, Blom et al. 1995, Van de Weerd et al. 1996, Van de Weerd et al. 1998, Beattie et al. 1998, Morisse et al. 1999, Harri et al. 2000). Although popular, this technique has limitations (Duncan 1978, Hutson 1984). There are concerns regarding confounding factors, such as prior experience, or unforeseen variables that may influence choice (Dawkins 1976, Cooper and Nicol 1991, Arnold and Estep 1994). Ultimately, different environments may fulfill different needs at different times, thereby making interpretation difficult. Still, preference testing can be useful, especially when combined with other types of research (Duncan 1992, Fraser et al. 1993, Fraser and Matthews 1997).

A similar preference technique is commonly used in laboratory experiments investigating choice strategies and how they relate to habitat selection in the wild. In this context there are some examples of preference testing in non-mammalian vertebrates (Webster 1986, Heinen 1993). However, there are no known studies with regards to the welfare implications of captive reptile environments. Logic suggests that if animals make specific choices, which ultimately influence their welfare (i.e. survival and reproductive success) in the wild, similar choices would likewise influence their welfare in captivity.

Box Turtle Natural History

The range of the eastern box turtle extends from southern Maine to Florida in the east and from Michigan to Texas in the mid-west. (Ernst et al. 1994, Gould 1998). The box turtle is terrestrial and typically inhabits open woodlands, but may also be found in marshy meadows or grasslands (Reagan 1974, Williams and Parker 1987, Ernst et al. 1994, Hall et al. 1999). Reported home range sizes for the eastern box turtle are quite variable, but usually encompasses a 100 – 175 m long elliptical area (Strang 1983, Williams and Parker 1987, Stickle 1989, Gould 1998). Box turtles are diurnal with little to no activity occurring at night (Ernst et al. 1994). While generally solitary animals, they have been reported feeding near one another without incident (Stickle 1950). However, males have been observed in combat-like behavior including ramming, biting and pursuit (Grace 2000).

Turtles are ectotherms and rely on the environment, or more importantly the microenvironment, to maintain appropriate body temperature (Pough 1991, Flanagan 1992, Warwick 1995, Gould 1998). To regulate temperature and hydration turtles will soak in water, bask in the sun or burrow into leafy debris. These shallow burrows are called resting forms and usually incorporate the superficial leaf litter and the top 1-3 cm of soil (Stickle 1950, Strass et al. 1982). Resting forms may also be used simply to avoid detection.

The environment and microenvironment also influence travel and habitat selection. Generally, box turtles are more active during warm days with high humidity and after periods of rain (Stickle 1950, Reagan 1974, Strang 1983, Penick et al. 2002). Daily movements can vary greatly from 0 to more than 100 m/day (Strang 1983, Penick et al. 2002). Box turtles have been known to remain hidden for several days or weeks, even when conditions are favorable for travel (Stickle 1950, Lemkau 1970, Penick et al. 2002).

Box turtles usually hibernate through the winter in burrows. These hibernacula are usually deeper than resting forms (up to 16 cm), with depth depending on the severity of the winter (Wetmore 1920, Dolbeer 1971, Claussen et al. 1991, Gould 1998). In the southern portion of their range, box turtles will leave their hibernacula during warm periods (Dolbeer 1971, Congdon et al. 1989). Captive box turtles do not need to hibernate if they are well-hydrated and maintained at room temperature (Ernst et al. 1994).

Box turtles are omnivorous. Their diet includes fungi, invertebrates, flowers and fruits (Strang 1983, Stuart and Miller 1987, Flanagan 1992, Ernst et al. 1994, Gould 1998). In captivity box turtles will eat a variety of items including canned dog food, earthworms, mealworms, and a wide range of fruits, and vegetables (de Vosjoli and Klingenberg 1995, Wilke 1998). Captive box turtles should be fed at least three times per week (Flanagan 1992).

Most captive situations cannot practically accommodate all the behaviors box turtles exhibit in the wild, especially typical travel habits. However, it is feasible to provide features that would encourage certain common behaviors. For the box turtle, materials for hiding and burrowing could easily be provided. The following chapters explore the potential benefits of such environmental enrichment in captive eastern box turtles.

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Chapter 2. Captive Habitat Preferences of Eastern Box Turtles.

Abstract

Appropriate housing conditions for captive reptiles have not been adequately evaluated. The purpose of this study was to determine if captive eastern box turtles (*Terrapene carolina carolina*) exhibit a preference for an enriched or barren environment. Thirty-eight box turtles were individually tested in a preference test enclosure, in which they could move freely between two environments. Relative dwelling time (RDT) was determined for each of the housing conditions. Turtles showed a distinct preference for the enriched environment (80.7% RDT, $p<0.01$). These turtles were then housed in either an enriched or barren enclosure for one month and reevaluated for preference to determine if previous housing experience affects choice. The turtles continued to prefer an enriched environment, regardless of prior housing conditions (97.6% RDT, $p<0.01$).

Introduction

The relatively recent rise in public concern for animal welfare has led to the improvement of the housing conditions for a wide variety of captive animals (Mench et al. 1998, Olsson and Dahlborn 2002, Roder and Timmermans 2002). Despite this heightened awareness, reptiles have been relatively disregarded. There has been a long held misconception that reptiles are stoic, adaptable and highly tolerant of extreme conditions. Since reptiles have strong innate drives and are not subject to parental and social education, they may be less tolerant and less adaptable to the unnatural environment of captivity (Warwick 1990, Warwick et al. 1995, Arena and Warwick 1995). As reptiles become increasingly popular as pets, it is important to address their captive welfare needs. In addition to providing the basic biological requirements of food, water, temperature and humidity, other attributes of the physical environment should also be considered in order to optimize well-being.

Preference testing is a popular approach to evaluate enrichment features of captive environments. This method of simply allowing an individual to choose between two or more housing conditions has been used extensively with laboratory (Arnold and Estep 1990, Arnold and Estep 1994, Blom et al. 1995, Van de Weerd et al. 1996, Van de Weerd et al 1998) and farm animals (Hughes 1976, Dawkins 1976, Dawkins 1977, Van Rooijen 1983, Morisse et al. 1999, Harri et al. 2000). While this technique is not without its critics (Duncan 1978, Hutson 1984), it has proven a useful tool in enclosure design and husbandry decisions (Broom 1988, Duncan 1992, Fraser et al. 1993, Fraser and Matthews 1997).

The objective of this study was to determine if eastern box turtles prefer an enriched housing environment to a barren one. Since box turtles often excavate and occupy shallow burrows in leafy debris, fallen logs and brush (Stickle 1950, Lemkau 1970, Strass et al. 1982), I hypothesized that they would prefer an enriched environment, where they could perform these activities. In order to evaluate the effects of prior housing experience on choice, turtles were tested a second time after living in either an enriched or barren enclosure.

Methods

Thirty-eight eastern box turtles were included in this two-year study. Turtles were obtained from two sources. Group 1 (n=17) consisted of wild caught box turtles brought to the North Carolina State University College of Veterinary Medicine (NCSU-CVM) Turtle Rescue Team. Turtles included in this population were restricted to those that presented with unilateral or bilateral aural abscesses (n=9) or with minor or no injuries (n=8). Group 2 (n=21) consisted of previously captive turtles maintained in public or private collections. For analysis, these turtles were stratified into three populations; aural abscess turtles (A), wild healthy turtles (W), and captive turtles (C).

Turtles were entered into the study between the months of June and September. Eighteen turtles were entered in 2000 and 20 turtles in 2001 (Table 1). These turtles were maintained as part of a related study that examined stress relative to captive habitat type (Case thesis, Chapter 3). The initial preference test was administered within three days of turtle acquisition. Turtles then spent one month housed in either an enriched or barren enclosure as described in Chapter 3. At the end of this period the preference test was repeated.

The preference test enclosure consisted of a 78 x 35 x 30 cm plastic Rubbermaid® container (Fig. 1). The barren environment, comprising one-half (39 x 35 cm) of the enclosure consisted of a flat newspaper substrate. The enriched environment, comprising the other half (39 x 35 cm) of the enclosure, was covered with 2-3 cm of cypress mulch. The enriched portion also contained a hide box (a shelter made from a plastic flowerpot cut in half lengthwise) and shredded paper. A shallow dish of water (17.5 x 13.5 cm) was placed adjacent to and at the mid-point of one side-wall.

At the start of each preference test an individual turtle was placed in the center of the test enclosure. Each turtle was initially positioned under a cover, which consisted of an inverted plastic flowerpot. The cover was used to reduce any placement bias. After 10 to 15 seconds the cover was lifted and the turtle was left alone. Each turtle was recorded for 6 hours using a videocassette recorded (VCR) and video camera, which was positioned directly above the test enclosure. Later the recordings were viewed to assess environment occupied and behaviors exhibited.

Turtles were determined to be in the enriched or barren portion if ≥75% of the animal occupied that space. If an individual was not committed to one side by at least 75%, or if it occupied the water dish, the turtle was considered to be in neutral territory. Relative dwelling time (RDT) was calculated using minutes in each environment divided by total time videotaped. Signed rank analysis was performed to determine if turtle movement between the two environments was equivalent to random. Individually, a turtle was determined to have a preference if it spent at least 60% of the time in a given environment. This limit was defined as two standard errors (5%) above chance (50%) (Arnold and Estep 1994).

Activity budgets were assessed for the two environments. Activities were divided into three main categories; moving, resting, and escaping. Escaping was defined as any interaction with the enclosure boundaries, such as wall climbing, clawing or digging directed at the wall, pacing with at least one foot contacting the wall, or bumping the wall repeatedly with the head. Moving included all movement not considered escaping, such as walking, turning, climbing on the hide box, and digging. Turtles were also considered moving if the hide box or litter in which they were under was moving. Resting encompassed all time periods when the turtle's body remained stationary, whether or not the head was moving. Percent time performing an activity in each environment was calculated based on the total time spent in that environment. Time spent utilizing cover was also calculated.

Analysis

The pre-study preference test data were analyzed using the Wilcoxon signed rank test. Occupation of the enriched environment was tested against random movement (50% occupation). Comparison of the pre- and post-treatment preference tests was accomplished with the Wilcoxon-Mann-Whitney rank sum test. Data for the Wilcoxon-Mann-Whitney were stratified based on source population of turtles. Two-sided p-values were calculated using StatXact™ software for SAS®. This software produces an exact test statistic, which is appropriate for small sample size. Differences were considered significant at p-values < 0.05.

Results

Pre-treatment preference

The pre-treatment preference test indicated an overall preference for the enriched environment ($P<0.01$). Turtles spent a median 90.9% RDT in the enriched portion of the test enclosure and 1.8% RDT in the barren half (Fig 2). Individual turtle preferences are presented in Table 1. Thirty-three of the 38 turtles (86.8%) had a pre-treatment preference for the enriched environment. Sixteen turtles (42.1%) spent no time in the barren environment. All turtles spent at least some time in the enriched environment.

Figure 3 illustrates the activities within each environment. Turtles spent a significantly greater percentage of time engaged in escape attempts while in the barren environment (median 46.1%) compared to the enriched (median 1.9%) ($p<0.01$). While in the enriched environment turtles spent 89.2% of the time utilizing cover provided by the hide box and shredded paper. Four turtles, by chance or intent, achieved cover under the paper substrate of the barren environment. Once under this substrate turtles often became more quiescent.

Post-treatment preference

There was no significant change in environmental preference test results following the one month housing study ($p=0.91$). Post-treatment preference results were similar to pre-treatment, with a median enriched RDT of 97.6% and 0.1% median barren RDT (Fig. 2). The overall preference for the enriched environment remained significantly high ($p<0.01$). Thirty-two of 38 turtles (84.2%) turtles showed a preference for the enriched environment, regardless of housing experience (Table 1). Nineteen turtles (50%) spent no time in the barren portion.

Activity distribution for the post-treatment preference test was similar to that of the pre-treatment test (Fig. 3). Box turtles spent a significantly greater percentage of time engaged in escape attempts while in the barren environment (median 40.5%) compared to the enriched (median 0.4 %) ($p<0.01$). While in the enriched environment turtles spent 97.5% of the time utilizing cover. Two turtles attained cover under the paper substrate while occupying the barren environment.

Discussion

These study results demonstrate box turtles have a significant preference for an enriched environment. The type of enrichment offered in this study mimicked that of natural turtle habitat. It seems intuitive that turtles would select conditions that resemble those to which they are accustomed, and these results support this.

Previous studies with chickens (Dawkins, 1977) and hamsters (Arnold and Estep 1994) have shown that prior housing experience can influence preference. This was not evident in this experiment. After spending time exclusively in a barren enclosure turtles continued to favor the enriched environment. The are several possible explanations for this. Turtles may not have been housed for a sufficient period of time to influence subsequent preference testing. The study duration was very short relative to the length of time these turtles have spent living in their natural environment, in the case of the wild caught turtles. Turtles acquired from collectors were housed in either outdoor enclosures or indoor enclosures similar to the enriched housing of this study. It is also possible that turtles may never have become accustomed to barren surroundings, regardless of the length of time housed in such conditions. If turtle behavior is truly “hard-wired” and not subject to change based on experience or learning (Warwick 1990) prior housing would not affect choice. This could be investigated through long term studies that maintain turtles in experimental conditions for extended periods of time, perhaps months or even years. Alternatively, turtle hatchlings could be raised in different housing conditions, to control for all early experience.

It is interesting to note that seeking cover seemed to be a common behavior and even when in the barren section turtles occasionally ended up under cover. This may have actually contributed to a higher barren RDT because, once beneath the paper substrate, movement and attempts to escape often ceased.

Results of this study demonstrate that an enriched environment is preferred over a barren environment. If choices are based on biological or psychological needs it seems logical that such choices would promote welfare. However, this can only be confirmed by studies correlating housing type with known stress indicators, such as immunosuppression, hormone production and behavioral changes. This study set out to answer a fundamental question, and the environmental choices offered were substantially different from one another. Follow-up studies are indicated to answer more specific questions, such as which aspects of the environment dominate during turtles' choice of environment.

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Table 1. Numbers of turtles entered into study per source population and year.

	wild abscess	wild healthy	captive	total
2000	7	5	6	18
2001	2	3	15	20
total	9	8	21	38

Table 2. Number of turtles that showed a preference for a particular environment (≥ 60% RDT in that environment). Includes pre-treatment and post-treatment results for barren-housed and enriched-housed turtles.

		enriched	barren	water	no pref
barren-	pre	18	2	0	0
housed	post	17	1	1	1
enriched-	pre	15	2	1	0
housed	post	15	2	0	1

Figure 1. Preference test enclosure with box turtle.

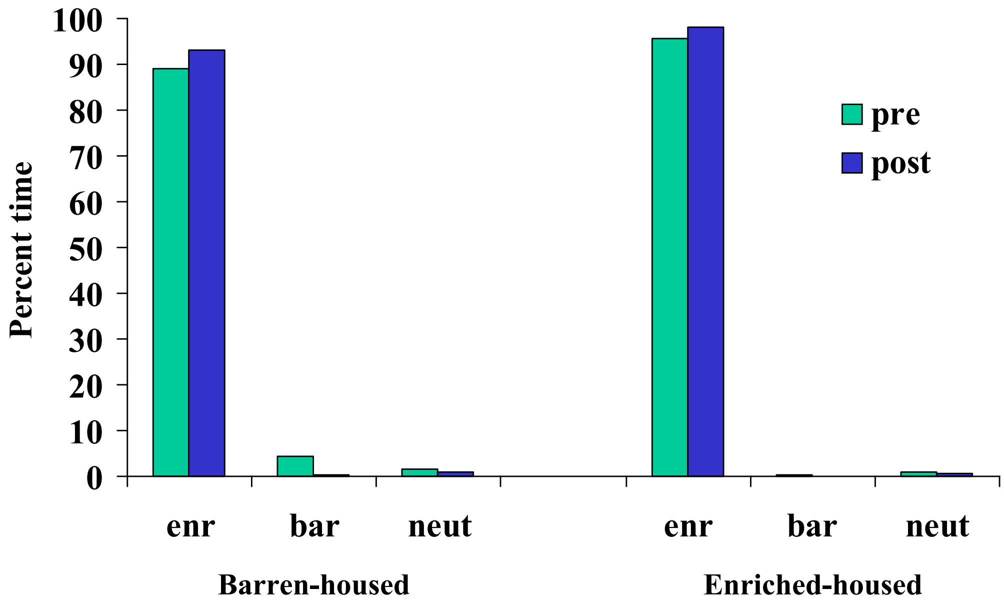

Figure 2. Median percent time barren-housed and enriched-housed turtles spent in different territories of the preference test enclosure. Both pre-treatment and post-treatment data are presented. enr = enriched territory, bar = barren territory, neut = neutral territory.

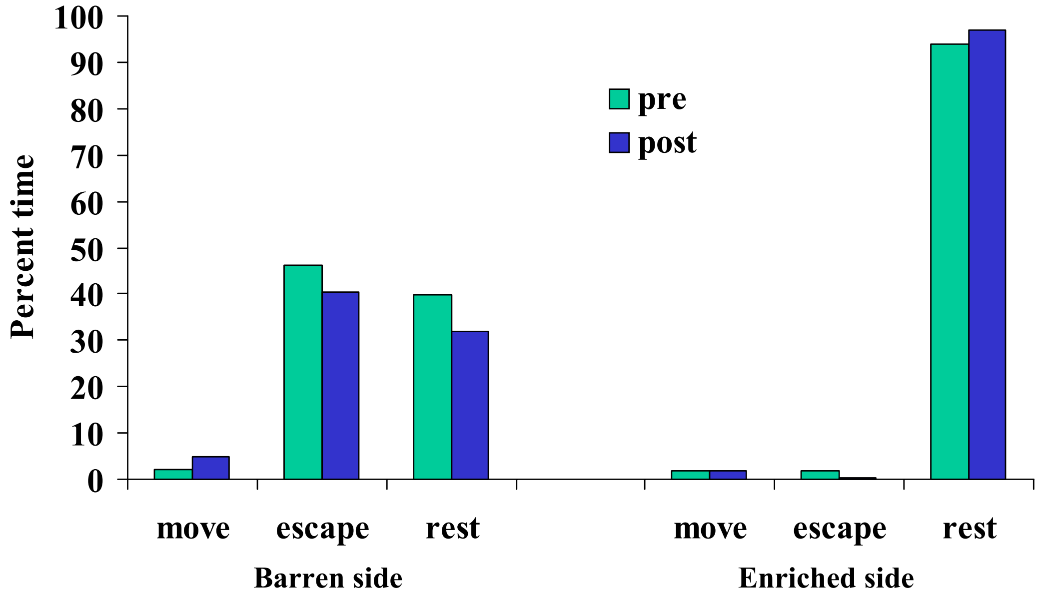

Figure 3. Median percent time box turtles spent engaged in various activities in relation to habitat occupied. Both Pre-treatment and post-treatment data are presented.

Chapter 3. Physiological and Behavioral Effects of Captive Housing Conditions on Eastern Box Turtles.

Abstract

Appropriate housing conditions for captive reptiles have been infrequently addressed. This study examines the physiological and behavioral impact of housing conditions on the captive eastern box turtle (*Terrapene carolina carolina*). Thirty-eight box turtles were randomized to either a barren or enriched enclosure for a period of one month. Complete blood counts, fecal corticosterone, and body weights were measured at the beginning and end of the study treatment period. Behavior was also assessed during the study. Turtles in barren enclosures had a significantly higher heterophil to lymphocyte ratio (H/L) at the end of the treatment period ($p=0.01$). Barren-housed turtles also spent more time engaged in escape behavior ($p<0.01$). Both physiological and behavioral measures indicate barren-housed turtles experienced higher levels of stress than turtles housed in enriched environments.

Introduction

The relatively recent rise in public concern for animal welfare has resulted in the study and improvement of the housing environments for a wide variety of captive animals (Segal 1989, Newberry 1995, Sheperdson 1998). Despite this heightened awareness, reptiles have been relatively disregarded in this process. There has been a long held misconception that reptiles are stoic, adaptable and highly tolerant of abnormal conditions. However, because reptiles have strong innate drives and are not subject to parental and social education, they may in fact be less tolerant and less adaptable to the unnatural environment of captivity (Warwick 1990, Warwick et al. 1995, Arena and Warwick 1995). As reptiles become increasingly popular as pets, it is important to address their captive welfare needs. In addition to providing the basic biological requirements of food, water, temperature and humidity, other attributes of the physical environment should also be addressed in order to optimize well-being.

There are numerous publications in the literature on the use of enrichment for mammals. A variety of farm, laboratory, and zoo animals, especially the non-human primates, have been extensively studied (Mellon et al. 1981, Wood-Gush and Beilharz 1983, Quick 1984, Markowitz and La Forse 1987, Pereira et al. 1989, Fraser et al. 1991, Carlstead et al. 1991, Fraser et al. 1991, Hubrecht 1993, Spoolder et al. 1995, Brinkman 1996, Lidfors 1997, Watson 1997, Cardinal and Kent 1998). While these studies provide a starting point for enrichment for animals in other taxa, they are not a substitute for such studies. In fact, these studies have proven the importance of considering the needs of the particular species (Wilson 1982, Perkins 1992), and in some cases the individual (Chamove and Anerson 1989, Line and Clarke 1989, Heinz et al. 1998), when designing enrichment programs.

Enriched housing, appropriate for the box turtle, should include materials that would enable turtles to perform behaviors commonly observed in their natural habitat. Box turtles often burrow into leafy debris, rotten logs, brush and soil. These shallow burrows are called resting forms and may function to regulate temperature and hydration or simply to avoid detection (Stickle 1950, Strass et al.1982, Strang 1983). Turtles have been known to remain hidden in these forms for several days or weeks, even when conditions are favorable for travel (Stickle 1950, Lemkau 1970).

Welfare assessment is an important part of any enrichment project. Common symptoms of stress include, anorexia, weight loss, elevated stress hormone levels and immunosuppression (McDonald 1976, Morton and Griffiths 1985, Broom 1988, Yosef 1988, Miller-Schroeder and Paterson 1989, Lance 1992, Rushen and de Passille 1992, Lance 1994, Chizar et al. 1995, Guillette et al. 1995, Clark et al. 1997, Terlow et al. 1997). Corticosterone is the primary stress hormone produced by reptiles (Lance 1990, Lance 1994, Guilette et al. 1994). The heterophil to lymphocyte ratio (H/L) is an effective measure of immunosuppression and has proven a reliable indicator of stress in several avian and reptilian species (Gross and Siegel 1983, Maxwell and Robertson 1998, Puvadolpivod and Thaxton 2000, Elston et al. 2000, Parga et al. 2001, Scope et al. 2002, Morici et al. 1997, Lance and Elsey 1999, Work et al. 1999, Work et al. 2001).

The objective of this study was to determine if turtle health and well-being are affected by housing conditions. Turtles, housed in either barren or enriched enclosures, were monitored for physiological and behavioral signs of stress. I hypothesized that an enriched enclosure, which mimics natural conditions, would improve box turtle welfare by reducing stress.

Methods

Thirty-eight eastern box turtles were entered into the study between the months of June and September. Eighteen turtles were entered in 2000 and 20 turtles in 2001 (Table 1).

Turtles were obtained from two sources. Group 1 (n=17) consisted of wild caught box turtles brought to the North Carolina State University College of Veterinary Medicine (NCSU-CVM) Turtle Rescue Team. Turtles included from this population were restricted to those that presented with unilateral or bilateral aural abscesses (n=9) or with minor or no injuries (n=8). Group 2 (n=21) consisted of previously captive turtles maintained in public or private collections. For analysis, these turtles were stratified into three populations; aural abscess turtles (A), wild healthy turtles (W), and captive turtles (C).

All turtles were individually housed at the NCSU-CVM lab animal resources' out-building facility in 50cm x 28cm x 30cm deep plastic containers. The study room was maintained on a 12:12 h light:dark cycle and temperature was maintained at 20-25°C. Enclosures were monitored for humidity and misted as needed to maintain the recommended humidity of 50-80% (Gould 1998). Turtles were fed 2% of their body weight every 48 hours with a diet consisting of dry puppy chow soaked in water, peas, corn and berries. These food items were selected based on ease of ingestion assessment due to their discrete nature. Other food items were added if turtles failed to eat after three consecutive feedings. These supplemental items included canned cat or dog food, shrimp, and live prey (mealworms, night crawlers, and earthworms). Turtles were placed in shallow water for 10 minutes every 48 hours to ensure hydration by encouraging drinking.

Turtles were randomized to one of two housing conditions, either barren or enriched. The barren environment included a flat paper substrate and a shallow water dish. The enriched environment consisted of cypress mulch substrate (2-3 cm deep), shredded paper, a hide box constructed from a plastic flowerpot cut in half lengthwise and a water dish. Turtles spent one month in their assigned environment and then were returned to their owners or released.

Body weight, heart rate, and food consumption were monitored throughout the study period. Body weights were measured using a triple beam balance. Heart rates were monitored using a doppler blood flow monitor. The probe was placed cranial to the right hind leg and the audible blood pulsation was counted over time. Early in the study heart rate was determined to be a poor indicator of stress due to the influence handling had on this variable (Smith and de Carvalho, Jr. 1985, Cabanac and Bernieri 2000). However, heart rate monitoring was continued for all turtles to maintain consistency of handling throughout the study.

Complete blood cell counts (CBC) and fecal corticosterone levels were determined at the beginning and end of the study. Blood (0.3 to 0.5 ml) was collected with a 25 gauge needle and 1.0 ml heparinized syringe via venipuncture of the brachial vein or the cervical sinus. CBC analysis was performed by the NCSU-CVM clinical pathology laboratory. Heterophil to lymphocyte ratios were calculated based on cell count results.

Fecal material was collected from the individual enclosures. The first available feces were collected as the pre-treatment sample and the post sample was collected within the last four days of the study period. Fecal samples were sent to the St. Louis Zoo’s endocrinology lab for corticosterone analysis. Fecal samples were collected only during the second study year.

For turtles with ear abscesses, time to complete recovery was determined. Complete recovery was defined as total absence of drainage or soft tissue swelling around the incision site, ears and head.

Behavioral observations (via video recording) were performed two weeks after turtles were placed in their new environments on two consecutive days (one feeding and one non-feeding day). Recording began between 0730 h and 0900 h and continued for six hours without interference. Videos were subsequently viewed and continuous behavioral data were collected for the entire recorded time. Activity budgets were determined based on these major categories; escaping, moving, resting and ingesting.

Escaping was defined as any interaction with the enclosure boundaries, such as wall climbing, clawing or digging directed at the wall, pacing with at least one foot contacting the wall, or bumping the wall repeatedly with the head. Moving included all movement not considered escaping, such as walking, turning, climbing on the hide box, and digging in the substrate. Turtles were also considered moving if the hide box or litter in which they were under was moving. Resting encompassed all time periods when the turtle’s body remained stationary whether or not the head was moving. Ingesting was designated as striking at, tearing or ingesting food or when the head or mouth broke the water surface. Percent time performing an activity was calculated based on the total observation time. Time spent utilizing cover was also determined.

Analysis

Comparison of the pre and post treatment parameters was accomplished with the Wilcoxon-Mann-Whitney rank sum test. Data for the Wilcoxon-Mann-Whitney tests were stratified based on source population of turtles. Correlation analysis was performed with the Kendall test. Binomial data were analyzed using homogeneity and odds ratio tests. Paired data were examined with the Wilcoxon signed rank test. Two-sided p-values were calculated using StatXact™ software for SAS®. This software produces an exact test statistic, which is appropriate for small sample size. Differences were considered significant at p-values < 0.05.

Results

Physiological

Figure 1 shows the median pre-treatment and post-treatment H/L ratios for both barren-housed and enriched-housed turtles. Barren housing did not change the post-treatment H/L ratio relative to pre-treatment values (p=0.95). Enriched housing resulted in a statistically significant decrease in H/L ratio from pre-treatment (median = 0.8381) to post-treatment (median = 0.3087) (p=0.04). The post-treatment H/L values for barren-housed turtles and enriched-housed turtles (0.8302 and 0.3087, respectively) were also significantly different relative to each other (p=0.01) (Figure 2).

Pre and post treatment fecal samples were available for 15 turtles. Changes in corticosterone levels were not notably different between the two housing groups (p=0.20) (Figure 3). There were 32 data points available with both H/L and corticosterone values.

These data were evaluated with the Kendall test for correlation, which was not significant ($p=0.68$).

Thirty turtles (78.9%) gained weight during the study period (range = 2 to 72 g, median = 18 g) ($p<0.01$). This weight gain represented an average 5.6% increase from starting values. Eight turtles (21.1%), lost weight (range = -1 to -29 g, median = -8 g) seven of these eight were in the captive population. Wild turtles (abscess and healthy combined) gained substantially more weight (median = 31 g) than the previously captive turtles (median = 9 g) ($p=0.01$) (Figure 4). There was no difference in weight gain based on treatment housing ($p=0.33$).

Nineteen turtles (50%) required the addition of non-standard food items. Seven of these ate little or nothing throughout the study. There was no distinction between treatment groups regarding reluctance to eat the standard diet ($p=0.48$).

Aural abscess healing time was assessed for eight turtles. There was no difference observed in healing time between the two housing groups ($p=0.96$). However, inconsistencies in abscess treatment (i.e. the use of antibiotics in some turtles) and disparity of elapsed time from abscess treatment to study entry may have compromised these data.

Behavior

Activity budgets, derived from over 450 hours of behavioral observation, are presented in Figures 5 and 6. Turtles living in enriched enclosures spent greater amounts of time resting and performing non-escape movement relative to barren-housed turtles ($p<0.01$ and $p=0.01$, respectively). Barren-housed turtles spent more time engaged in escape behavior than enriched-housed turtles ($p<0.01$).

Turtles living in enriched housing spent 91% time concealed within the hide box, litter or mulch. While hiding material was not available to barren housed turtles, 14 of 20 turtles (70%) still managed to find cover during the observation period, by crawling under the water dish or the flat newspaper substrate. Once under the paper movement and escape attempts often ceased.

Figure 7 illustrates the change of activity pattern for feeding versus non-feeding days. Paired data analysis revealed barren-housed turtles attempted escape more often on the day they were fed compared to the day they were not ($p=0.04$). These turtles also spent significantly less time resting on the day they were fed ($p<0.01$). Food presentation did not appreciably change the activity pattern of the enriched-housed turtles.

Discussion

There is both physiological and behavioral evidence that turtles benefit from an enriched environment. The substantially decreased H/L ratio observed in the enriched-housed turtles indicates they experienced less stress than barren-housed turtles. Similarly, the marked difference in activity budgets, especially escape attempts, suggests the enriched-housed turtles were more accepting of their housing conditions.

The impact of housing on immune function, which is reflected by the difference in H/L ratios, has important implications for turtles maintained in long-term captivity. For turtles kept as pets or in educational exhibits, housing conditions may ultimately influence health and longevity. For wild turtles undergoing rehabilitation due to illness or injury, housing may be an important factor affecting healing time. Differences in healing time were not evident in this study, however this may be due to the small sample size and the dissimilarity of treatments. The impact of enrichment on healing should be investigated further with standardized treatments and greater numbers of turtles.

A number of studies have demonstrated stress induced H/L changes are due to increased corticosteroids (Morici et al. 1997, Laakko and Fraker 2002, Webster et al. 2002). This study revealed neither a difference in corticosterone between treatment groups nor a correlation between H/L ratio and corticosterone concentration. Reduce corticosteroid production due to chronic stress has been reported in rodents (Mar Sanchez 1998), alligators (Lance and Elsey 1999) and pigs (de Jong et al. 2000). In addition, the glucocorticoid pathway is not the only mechanism of stress-induced immunosuppression, endogenous opioid peptides have also been implicated (Wang et al. 2002).

The higher weight gain observed in the wild population versus the captive could be due to several factors. Turtles in the wild must expend energy to forage, and food may be scarce. Presumably, while in captivity these turtles expended less energy obtaining food and the study feeding regime met or exceeded the caloric requirements of captivity. The previously captive turtles already experienced a similar feeding routine and therefore were less likely to gain as much weight.

The reason for the altered activity budgets of barren-housed turtles observed on feeding days is not known. One possible explanation is that the alternate feeding days always corresponded with the 48 hour soaking schedule. Soaking was always performed in the morning. Perhaps movement and therefore, escape attempts, were stimulated by soaking or the additional handling associated with this procedure. A slight, though non-significant, increase in movement was also observed in enriched-housed turtles on these days.

The behavioral results of this study also advocate enclosure enrichment for captive box turtles. The enriched environment enabled turtles to perform natural behaviors, such as digging and hiding, and decreased escape behavior. This study indicates that hiding materials are an important aspect of the box turtle’s environment. When available these hiding places were utilized 91% of the time and even when not made available, turtles often created their own hiding places.

This study shows that housing environment can negatively or positively influence the physiology and behavior of box turtles. Relatively simple enclosure modifications, such as substrate and hiding materials ameliorate the stress of captivity and should be provided for optimal care of the captive eastern box turtle.

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Table 1. Numbers of turtles entered into study per source population and year.

	wild abscess	wild healthy	captive	total
2000	7	5	6	18
2001	2	3	15	20
total	9	8	21	38

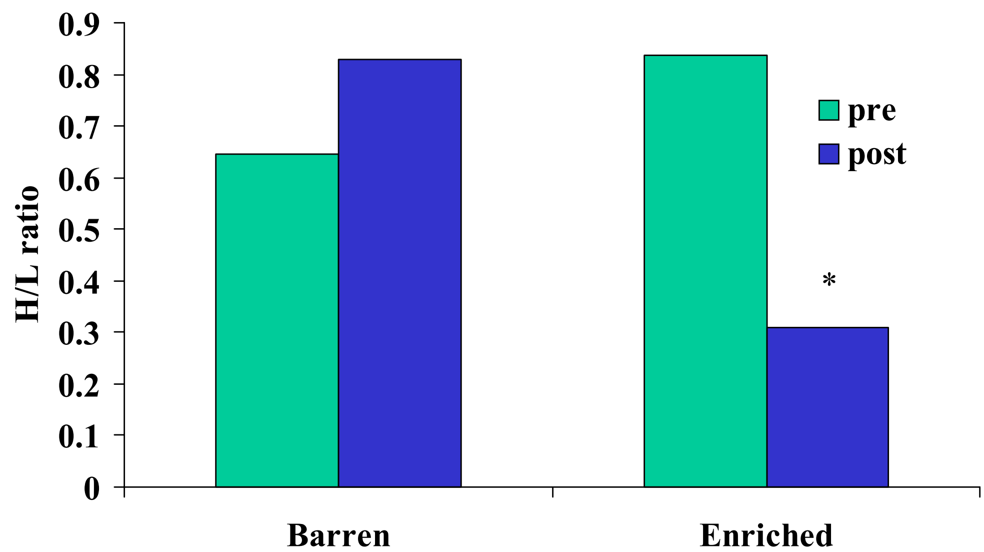


Figure 1. Pre-treatment and post-treatment median H/L ratios for barren-housed and enriched-housed box turtles. * significantly different from pre-treatment value (p=0.04)

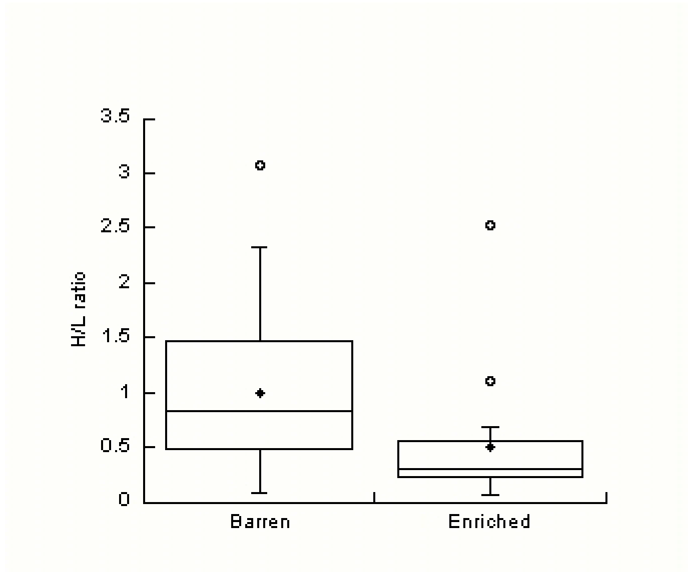


Figure 2. Box plot of the post-treatment H/L ratios for barren-housed and enriched-housed turtles (p=0.01).

The horizontal line of the box represents the median value of the data set, the top and bottom portions of the box encompass 25% of the data points above and below the median, respectively. The resulting box is the interquartile space. The open circles (o) represent data points that fall outside the acceptable range, which is defined as 1.5 times the interquartile space. These points are plotted as outliers, although all data points are included in the analysis. The upper and lower T-bars mark the maximum and minimum data points within the acceptable range. The solid diamonds (♦) indicate the mean. This description is the same for all the box plots that follow.

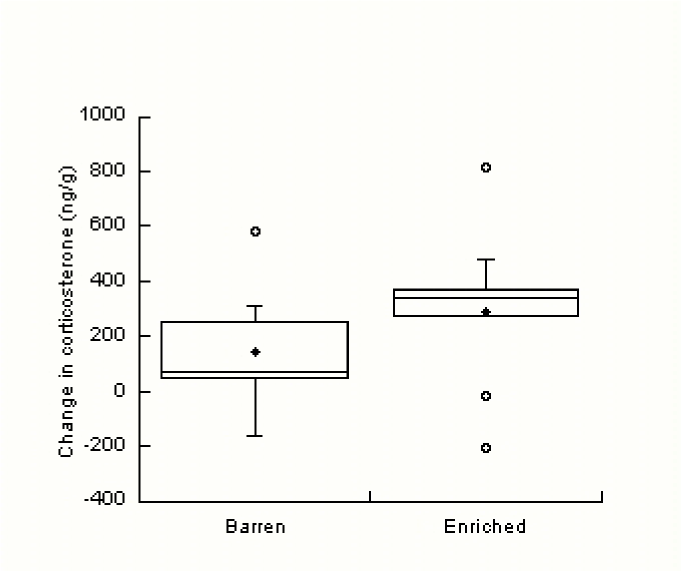


Figure 3. Change in fecal corticosterone levels of box turtles after living in either a barren or enriched enclosure for one month (p=0.20).

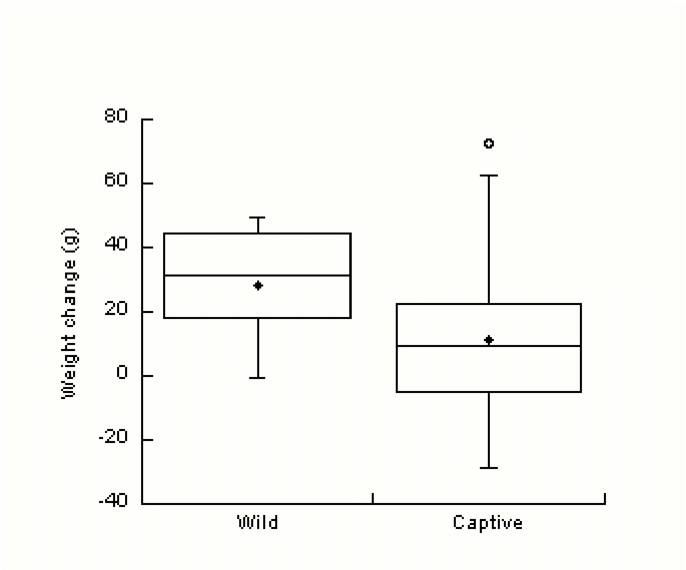
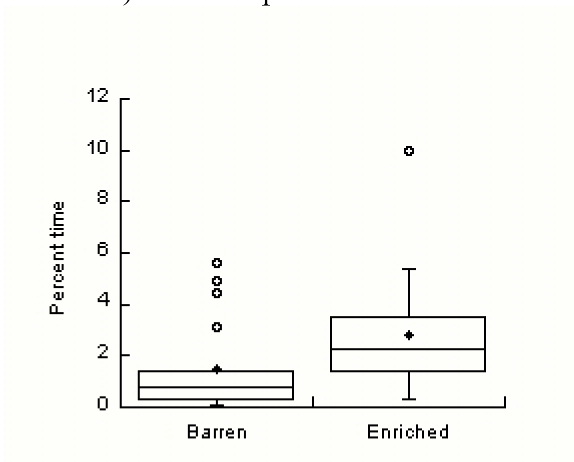


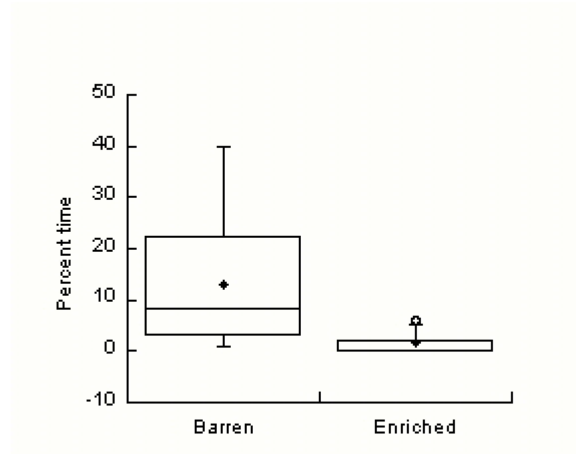
Figure 4. Post-treatment weight change for wild and previously captive box turtles (p=0.01).

Figure 5. Median percent time engaged in various activities for barren-housed and enriched-housed turtles.

A) Non-escape movement



B) Escape attempts



C) Resting

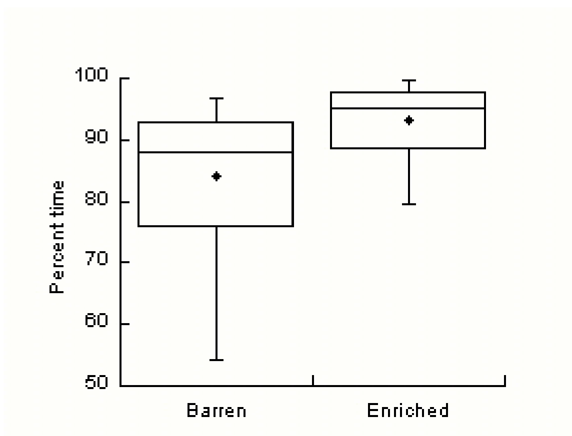


Figure 6. Comparison of percent time engaged in specific activities for barren-housed and enriched-housed turtles. A) Non-escape movement ($p=0.01$). B) Escape attempts ($p<0.01$). C) Rest ($p<0.01$).

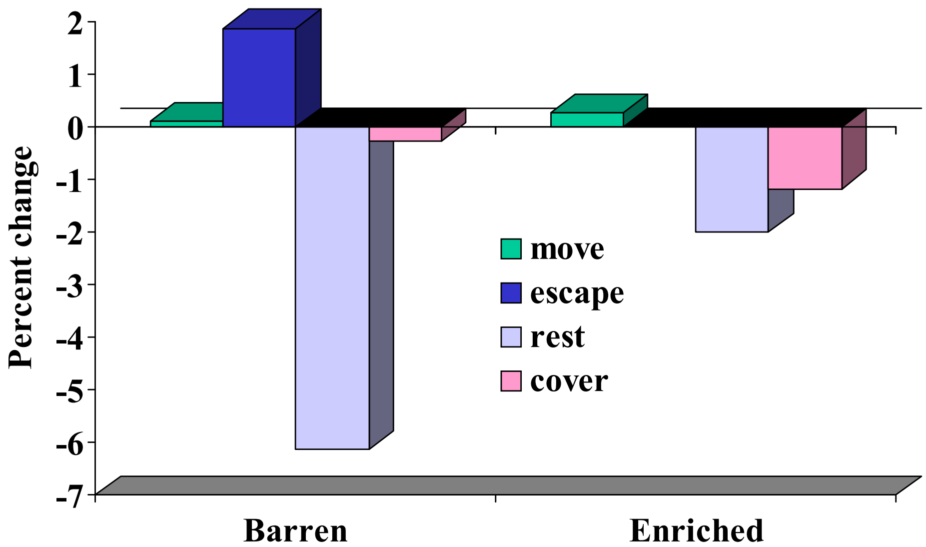


Figure 7. Median percent change in activity pattern for barren- and enriched-housed turtles.