CONSEQUENCES OF VARIATION IN DIETARY PROTEIN ON CAPTIVE-RAISED BLACK KNOB MAP TURTLES (*GRAPTEMYS NIGRINODA*, EMYDIDAE)

THESIS

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by

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San Marcos, Texas
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his data without question in order to further research on this sensitive genus. His unselfish willingness to help is refreshing and uplifting.

And very importantly, I would like to thank my friends and family. They heard more about hatchling and juvenile turtles than I think they ever wanted to know. Without their love and continued support, this would have been a failed project.

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ABSTRACT

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Many turtle species are threatened with extinction worldwide. Because of their slow maturation rates, low juvenile survivorship and slow population recovery status, more aggressive conservation tools including captive propagation may be required to prevent extinctions. Captive propagation is the practice of hatching eggs in an environment with fewer natural predators, better living conditions, and consequently decreased mortality.

Often adults are kept to create a "farmed population" and the juveniles are released into the natural habitat. However for any conservation tool to be effective, the released juveniles must have a realistic chance of survival and reproductive success in the natural environment. This study was designed to gather information about the potential consequences (e.g. growth and mortality) of variable protein diets on captive-hatched juvenile *Graptemys nigrinoda* (Black Knob Map Turtle). The experiment had two distinct parts: a 9-month exposure to one of three dietary proteins, followed by a 9-month exposure to the medium-level dietary protein. Over the variable portion of the experiment, all three diets had significantly different growth curves. After the 9-month medium protein level diet, all turtles, regardless of previous protein level, achieved the same end point. Importantly for propagation efforts, juvenile mortality does appear to be related to protein level; the highest dietary protein level sustained the fewest number of mortalities while the lowest protein had the lowest survivorship.

INTRODUCTION TO THE STUDY

The 285 extant species of turtles represent a unique lineage of biodiversity that has persisted over 220 million years (Gaffney, 1984). Turtles are classified by a collection of unique attributes. These attributes, or synapomorphies, easily distinguish turtles from all other organisms, and few argue the monophyly of turtles and their evolutionary history (Agassizi, 1857). All turtles have a skull lacking any cranial fenestra, and a protective shell. The shell is either bony or covered in leathery skin (Ernst and Barbour, 1992).

Turtles are increasingly more vulnerable to extirpation and extinction due to habitat loss and other anthropogenic factors. Human interaction, be it alteration of habitat or the use of turtles for food, has led to the need for conservation efforts for numerous turtle species across the globe.

Exacerbating this problem, policy makers appear to be indecisive in their approaches on how to formulate, implement, and/or execute those efforts. Roughly two-thirds (67 %) of all turtle species are on the IUCN Red List. The IUCN is a voluntary organization whereby its members agree to protect endangered species and their habitats (IUCN, 2003). In addition, nearly all turtle taxa (94%) are covered on the CITES appendices. CITES is the organization that specifically applies to trade of endangered species (CITES, 2004). Unfortunately, despite significant efforts by such international NGOs, management authorities, and turtle conservation organizations, turtle population

numbers continue to decline.

And while the rapid decline of numerous Asian species due to over harvest, primarily for food, appears to get the most attention in the popular press (the Asian Turtle Crisis) and is most often emphasized in documenting the decline in turtles, the reality is a global problem. Dwindling turtle populations are a worldwide phenomenon driving and leading turtle and tortoise species toward dangerously low population sizes throughout much of their distributions (Moll, 1976; Smith, 1979; Alho et al., 1985; Baard, 1989). Even though the situation in Asia is alarming, in reality, the leading cause of population declines remains habitat alteration through destruction and fragmentation (Diamond, 1986). Although the problem of loss of biodiversity appears to be focused in the tropical and terrestrial ecosystems of developing countries, other biomes and ecosystems are at risk as (Lydeard and Mayden, 1995). The International Union of Conservation of Nature (IUCN) lists 209 species of turtles and tortoises on its ever-expanding Red List (IUCN Red List, 2003). This list represents the most thorough and extensive list of vulnerable, threatened and endangered organisms, and while not all of these turtles are endangered *per se*, their trade and/or removal from their habitat pose a real and serious threat to them. Additionally, 269 species of turtles and tortoises worldwide are included in the CITES-listed species database (*UNEP-WCMC Species Database· CITES-Listed Species, 2004*). What we can gather from the CITES Species List and the IUCN Red List is that turtles as a group are in real danger of extinction and extirpation. Because of the slow life history (e.g. long maturation times) of these animals, the recovery time from over harvest and human interaction is incremental and arduous (Ernst et al., 1972; Frazer, 1986; Ernst and Barbour, 1989).

In North America, turtle population numbers follow the same negative trend as turtles around the world. Currently, the IUCN lists thirty-one (31) of the 52 species of North American turtles and tortoises on the Red List (IUCN Red List, 2003). While in other parts of the globe over-harvesting turtles for consumption is a major factor, in North America, the primary cause of declining numbers is habitat loss (Lydeard & Mayden, 1995). A particularly sensitive ecosystem of North America is the Southeastern United States, where the loss of wetland habitat threatens numerous aquatic and semi-aquatic species, not just turtles (Dahl, 1990). With the exception of tortoises, turtles are intimately tied to wetlands (Ernst, *et al.*, 1972), and with the reduction of those wetlands and marshes, turtles are increasingly confined to narrower and smaller available habitats. This is not to say that tortoises do not face their own unending list of factors influencing their demise, only that the focus of this study is aquatic turtles.

One area of specific concern is a portion of Southeastern United States, located primarily in Alabama (Fig. 1). This area, called the Mobile River Basin (MRB), represents a major drainage system for Alabama and its surrounding states, from the Appalachian Mountains to the northeastern portion of the Gulf of Mexico (Lydeard & Mayden, 1995). Lydeard and Mayden suggest that "extremely diverse" freshwater ecosystems such as the MRB are as important for conservation efforts as the terrestrial ecosystems of the tropics (1995). This fragile aquatic ecosystem supports a high level of diversity but currently has 32 aquatic plants and animals (including two turtles) covered by the Endangered Species Act of 1973 (USFWS, 2000; ESA Species List, 2004). While high, this number only represents the organisms covered by the ESA; organisms protected by individual states are not included in this number (Table 1). The reason for

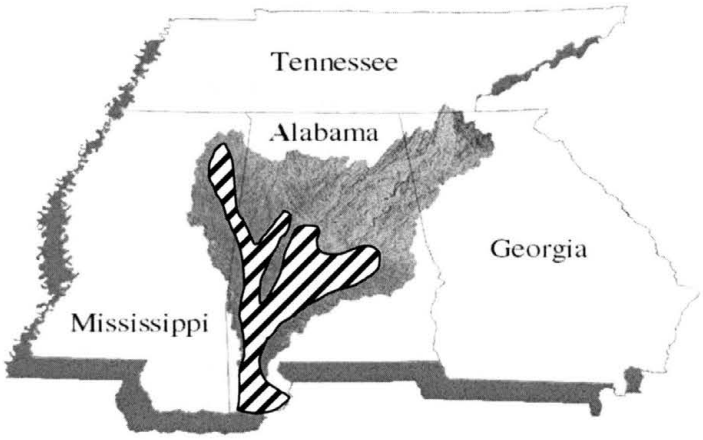


FIGURE 1. Map of the Mobile River Basin (MRB) drainage area within Southeastern United Sates including *Graptemys nigrinoda ssp.* distribution.
Modified from USGS map al.water.usgs.gov/ pubs/mobl/mobl.html

TABLE 1. Proportion of turtles covered by the Endangered Species Act (ESA) within United States and partitioned for each state in Mobile River Basin.

State of occurrence	# Turtles Covered by ESA	% Total ESA Turtles found in each state[1]	# of State Protected Turtle Species	% of Protected Emydid Turtles in each state of Total US Emydids[2]
AL	8 (1 Emydid)	61.5*	30 (14 Emydids)	45.2
GA	6 (1 Emydid)	46.2*	12 (3 Emydids)	9.7
MS	8 (2 Emydids)	61.5*	13 (5 Emydids)	16.1

* Taxonomic replication exists across these states

[1] – There are 13 turtles listed on the ESA.

[2] – There are 31 turtles under protection by individual states within the USA.

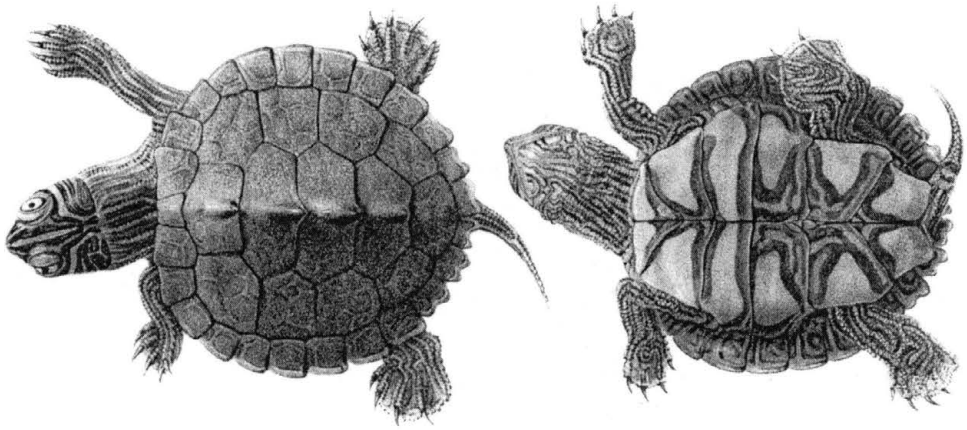
such high habitat fragmentation in the MRB is due, in part, to artificial impoundments on the natural waterways and rivers. These impoundments provide electric power to the people of Alabama and Mississippi at the additional cost of disrupting the local flora and fauna. In the early 1990's, U.S. Fish and Wildlife Service recognized a need for a recovery plan for the MRB. A plan was drafted and signed by U.S. Fish and Wildlife Service (2000). The plan focuses primarily on the aquatic portion of the Mobile River Basin and the organisms that inhabit those waters. While a plan is necessary to promote education and activism, the plan relies almost entirely on the efforts of volunteer organizations concerned about the status of the MRB.

One genus of aquatic turtle that resides almost entirely within the United States is *Graptemys* (Agassizi, 1857) or the Map Turtles. These turtles have a wide range from Texas to Florida and from the Gulf Coast north to Quebec, including the MRB. *Graptemys* are members of Emydidae, or basking turtles, and resemble other taxa in this family, such as *Trachemys* and *Chrysemys*; however, they do have several distinguishing characteristics from habitat and diet to morphology that make them their own distinctive group (Ernst, *et al.*, 1972). With twelve known species, *Graptemys* represent the most speciose genera in Emydidae (Lamb, *et al.*, 1994). Their body form is like that of other Emydids, stream-lined for movement through water, but they have a distinctive keel down the middle of the carapace. This keel is sometimes marked with rings (as in the case of *G. oculifera*), knobs (as in the case of the *G. nigrinoda*) or spines (as in the case of *G. barbouri*). There are two distinct clades of *Graptemys*, the broad-head group and the narrow-head group (Ernst, *et al.*, 1972). The different clades partition food and habitat resources, and members of several species can often be found in sympatry (Vogt,

1981). To date, eight (8) of the twelve (12) *Graptemys* species are under various threats that categorize them from endangered to near threatened and are classified as such on the Red List (IUCN Red List, 2003), and eleven (11) have either Federal or state protection (USACE, 2003). Most (9 of 12) of the species in *Graptemys* exhibit drainage endemism (Lamb, *et al.*, 1994). The declines for nearly all species in this sensitive genus are thought to be attributed to human interference, through over harvest for the pet trade and/or habitat fragmentation (Lydeard & Mayden, 1995; USACE, 2003).

The model for this study, *Graptemys nigrinoda* is native to the MRB. This turtle is not federally covered by the ESA, but is protected in the states where it occurs (Fig. 1). The IUCN has recognized the plight of this animal and has included it on the Red List as Lower Risk - near threatened (IUCN, Red List, 2003). *Nigrinoda*, Latin for "black knobs," describes the knobs that point straight off the carapacial keel that make *G. nigrinoda* distinguishable from other *Graptemys* species (Fig. 2). Like other *Graptemys*, these turtles are highly aquatic, preferring fallen logs and debris for basking sites. They are typically shy and elusive (Ernst, *et al.*, 1994), probably contributing to the shortage of data from the wild. And like other aquatic organisms, these *Graptemys* are sensitive and vulnerable to habitat destruction and degradation (Lydeard & Mayden, 1995: Richter, *et al.*, 1997; USFWS, 2000).

With the outlook so bleak, is there anything that can be done to reverse the trend of declining turtle numbers, not only across the globe but here in the United States as well? Conservation efforts have been focused for years on marine turtles and protecting their nesting sites from predators, weather devastations and anthropogenic effects. Some



A. B.

FIGURE 2. *Graptemys nigrinoda*. A. Carapacial view with distinguishing knobs on the raised keel. B. Plastron view of the same individual. The differences in the *G. nigrinoda* subspecies are characterized by the markings on the plastron, in addition to a few other characters (Agassizi, 1857).

conservation groups tout great successes with the marine turtles, but the "World-wide Turtle Crisis" extends beyond the marine turtles and "Lonesome Georges" of the world. Some conservationists believe that captive propagation might be an answer to this problem.

Captive propagation and head starting are conservation techniques that may be particularly useful in efforts concerning Type III survivorship organisms (Pearl, 1928) (Fig. 3). These organisms are characterized by high reproductive output (many offspring) and little or no survivorship at early stages, where life expectancy increases for those individuals who survive the juvenile stage (Pearl, 1928). Often, Type III curves are associated with r-strategists (MacArthur & Wilson, 1967). Such r-strategists are characterized by high number of poorly provisioned offspring, population size existing below the carrying capacity of the environment, and limited social organization (MacArthur & Wilson, 1967). Type III organisms appear to be particularly sensitive to population declines because of their life histories. These organisms rely on a relatively small percentage of adults to offspring in order to populate each generation. Should mechanisms be in place that reduces the number of reproductive adults even further from the population, there might be too few individuals remaining to compensate for the decreasing numbers.

Captive propagation and head starting are designed to help turtles and other Type III survivorship organisms survive past the high mortality cliff associated with the hatchling to juvenile life stages. Typically, this stage of life experiences the highest mortality compared to later life stages. Once reaching adulthood, a turtle has a relatively

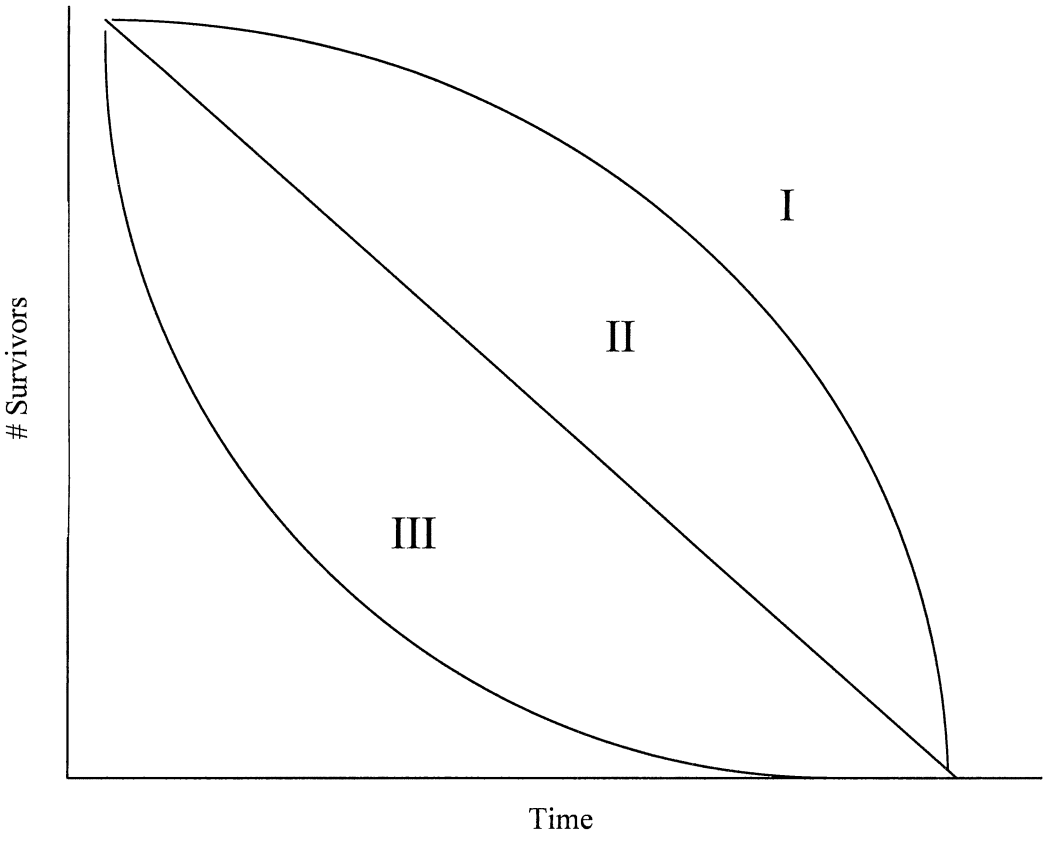


FIGURE 3. Survivorship curves for Type I, II, and III organisms as described by Pearl in 1928. Turtles are reported to exhibit Type III survivorship curves, characterized by little or no low probability of survival at early stages, and relatively stable mortality thereafter.

good chance of long-term survival. There are various kinds of captive propagation including, but not limited to, removing eggs from the wild, incubating eggs in a safe environment, and using wild-caught turtles to start a "farmed" population. The objective for captive propagation is to release at least a portion of the offspring back into the wild with the intent of increasing the wild populations' numbers. The longer the hatchlings are head-started, the more individuals survive the high-mortality juvenile stage.

One advantage to captive propagation might be that the offspring released into the natural habitat represent the organism's best chance for long-term survival. Like other Type III survivorship curve animals, turtles that survive past the high mortality of the juvenile stage have a good chance of reaching adulthood. As has been the case in alligators and marine turtles, captive propagation has increased the number of juveniles in the population. Therefore, captive propagation might have the ability to shift the survivorship curve of Type III organisms more towards Type II or even Type I by increasing the mean juvenile survivorship. However, in order for captive propagation to be effective, research and development to determine the optimal growing conditions are required. This level of commitment in time and resources can be expensive. Additionally, there are considerable expenses involved with housing and feeding hundreds, maybe thousands of hatchling and juvenile turtles. I was given an opportunity to explore the effects of dietary protein on the growth of hatchling *G. nigrinoda ssp.* in order to determine a "most-beneficial" level with the intent of changing or adapting current captive propagation techniques.

This experiment provides valuable information for several reasons. First, little empirical data have been gathered on the elusive *G. nigrinoda.* Also, no published

research has conducted a long duration study of the effects of variable protein levels on juvenile emydid growth and mortality. The present study was conducted on *G nigrinoda ssp* that could benefit from captive propagation, or head-starting techniques. This experiment can be used as a preliminary guide to protein and dietary needs for these sensitive turtles. I attempted to answer the following questions with this research: 1) Does dietary protein affect the growth of omnivorous/carnivorous hatchling turtles? 2) Is any effect of dietary protein sustainable once the juvenile is no longer subjected to the variable protein portion of the experiment? 3) Can dietary protein be correlated to juvenile mortality? 4) Are there any predictions about size and growth of *G. nigrinoda* based on the growth curves generated by Lindeman (1999) for other *Graptemys spp*?

EXPERIMENTAL DESIGN

Pond

A man-made pond 2.4 m (length) x 1m (wide) x .6 m (high) was constructed in order to raise over two hundred hatchling turtles at one time. This pond provided a stable environment with constant temperature and water conditions as well as shelter from the outside. A temperature-controlled lab, located within 10 miles of the university campus, provided superior security for these rare turtles.

The frame of the pond was constructed of lumber purchased at a local hardware store and EPDM rubber lining. Upon completion, the 0.85 m^3 (225 gallon) pond consisted of six (6) equal volume bins including a separate compartment for the water filtration and return system (Fig. 4A). The bins were separated by sturdy plastic mesh, with openings large enough to allow water flow, but small enough to contain food particles and turtles within each bin. Each bin had its own crawl-out space and heat/light source for basking (Fig 4B). The turtles in each bin were secluded from turtles in all other bins at all times.

The pond used a premium-grade 450-gal/hr pump to ensure maximum productivity and long life. A complete filtration system was constructed to filter the water and maintain high water quality. The filtration system was constructed from PVC tubing that was shredded to provide supporting media for biological filtration. These

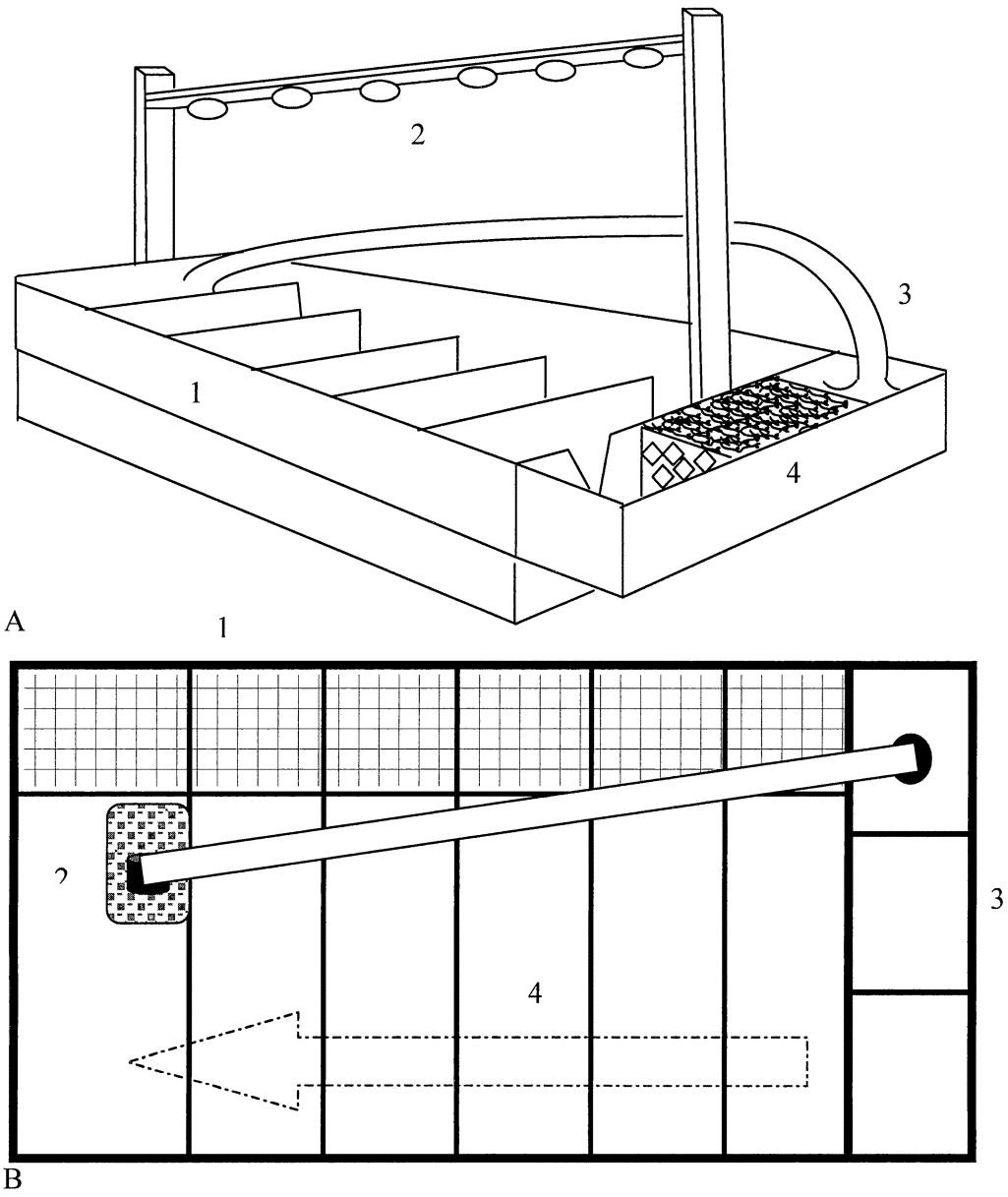


FIGURE 4. Man-made pond consists of 6 equal-volume bins. Rigid plastic screen was used to construct the partitions within the pond so that water could flow through, but turtles and food could not. A. Side view showing 1. Partitioning into the different bins. 2. Lighting source for basking. 3. Return water system (pump and equipment). 4. Rapid biological filtration system. B. Overhead view showing 1. Above-water basking areas 2. Return water system (pump). 3. Filtration system. 4. Direction of water flow.

coils provided rapid purification of the water and were lightweight, sturdy, and could be easily cleaned.

Turtles

This experiment was performed under IACUC # 7e48XG_02 for the duration of this experiment.

Both subspecies of *Graptemys nigrinoda*, *G. n. nigrinoda* and *G. n. delticola*, were used for this study; however, there was no consideration as to the number or ratio of *G. n. nigrinoda* to *G. n. delticola*. Guthrie Turtles donated over 400 hatchling turtles for each experiment, and those hatchlings shipped via Airfreight from the Concordia farms, in Louisiana after permits were issued by M. Lea, Jr., D.V.M. of the Louisiana Dept. of Ag and Forestry. Upon arrival, the hatchlings were sorted randomly and equally into the 6 bins of the pond. When the experiment began, there were no less than 30 turtles / bin. Each turtle was individually marked, including a cohort number, to distinguish all turtles (Fig. 5). The markings were in accordance with normal turtle markings and were conducted by clipping the marginal scutes that corresponded to the correct number for each turtle.

Dietary Protein Level

The food used in this study was ARKAT, Inc. dry floating pellets. There were three (3) diets in this study: High (42% crude protein level), Medium (32% crude protein level) and Low (14% crude protein level); here after referred to as 42, 32, and 14, respectively.

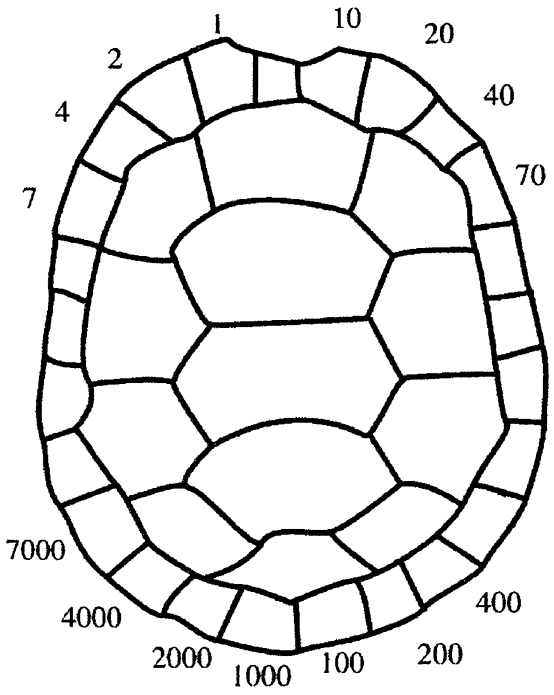


FIGURE 5. Marking system for juvenile *Graptemys nigrinoda* for the evaluation of dietary protein effects on these turtles. Each turtle was marked with an individual pattern clipped into the marginal scutes using nail clippers. For example, to mark Turtle # 57, clip the 1st and 3rd marginal scute to the right of the nuchal and the 4th marginal scute to the left.

Coordination with ARKAT, Inc., the manufacturer of the pellets, insured the same ingredients for all three feed-types. ARKAT also generously donated the feed required for this project. The diets differed in protein content only. There were two (2) bins per dietary protein level, providing replication in the study, for a total of six (6) bins. (Fig. 6).

Duration

The study was performed on two cohorts. The first cohort began Nov 2002 and completed Jul 2003; the second cohort began in Sept 2003 and completed Apr 2004. There were 9 measuring periods consisting of four-week intervals for each experiment. The turtles were fed only one of the three diets for the duration of the experiment. At the conclusion of each experiment, all surviving turtles were relocated to a bin consisting of a uniform diet at 32% crude protein. At the termination of 9 additional measuring periods, the turtles were measured a final time.

Care

At the beginning of each experiment, the turtles were fed fresh Romaine lettuce for one week. This week served as an acclimation period for the turtles. The turtles were not technically considered in the program at this time. The experiment began when the turtles began eating their specified diet.

The water in the tank flowed continuously. There were weekly water changes to remove excess protein, any unincorporated food and debris build-up. On measurement dates, entire water changes were performed, using a high-powered vacuum to remove any and all possible debris.

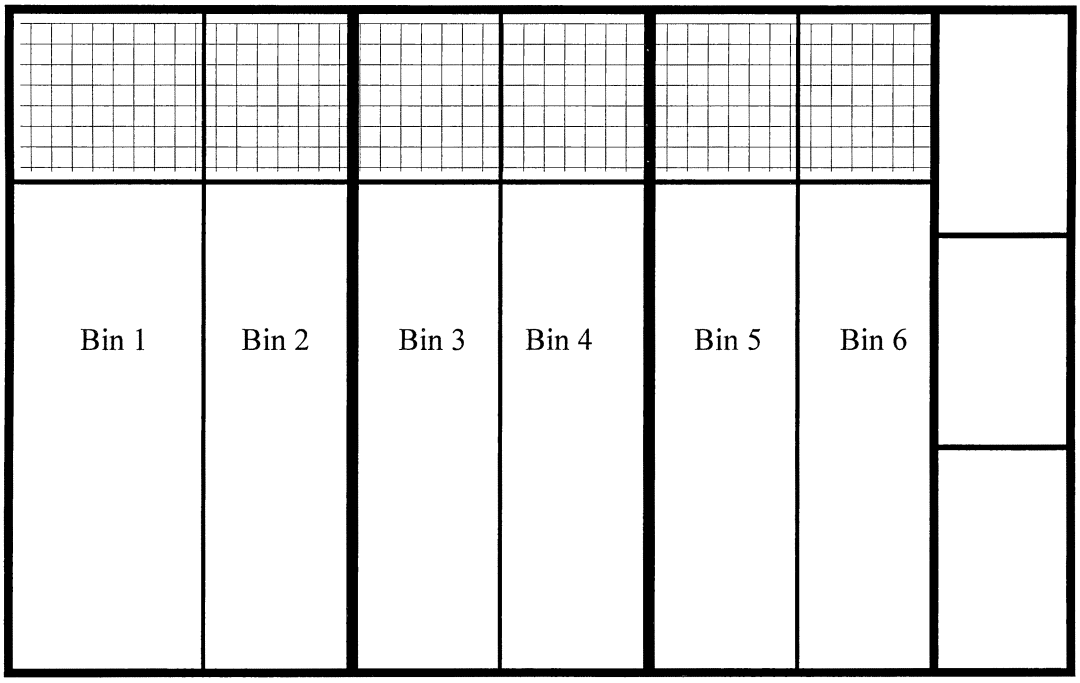


FIGURE 6. Pond design and layout for the evaluation of dietary effects on juvenile *Graptemys nigrinoda*. Juveniles in Bins 1 and 2 were fed the high protein (42%) diet; Bins 3 and 4 were fed the medium protein (32%) diet; Bins 5 and 6 were fed the low protein (14%) diet.

Feeding

The amount of food allotted per day for each bin was dependent on the mass of all turtles in each bin. The amount of food was calculated as:

Mass food/day = [(total mass turtles in bin) *0.05]/2

This corresponds to 2.5% of the total weight of turtles in the bin. The pellets were weighed using a digital gram scale. Because it would be time-consuming and error-prone to weigh the pellets everyday for feeding, the amount of food allocated for daily feeding for each growth period (time between measurements) was measured using teaspoons and tablespoons to get a dry volume of the pellets. This amount corresponded to recommended feed amounts by the manufacturer (2.5% of total body weight), which has been used in similar studies (Parmenter & Avery, 1990; Nuangsaeng & Boonyaratapalin, 2001). The amount of daily food was proportionally constant to turtle weight during this period, increasing as new measurements revealed increases in total turtle weight of each growth period. If the turtles increased in mass, the amount of food for the bin increased.

There was no consideration for competition within the six bins. Likewise, there was no consideration for feeding to satiation. Therefore, there was no mechanism in place to ensure that all turtles in each bin ate equally.

The results from this study were compared to other growth studies for *Graptemys* (Lindeman, 1999) at several ages for the juveniles, taking advantage of existing growth data to provide context for the results.

Measurements and Data Collection

Data were collected in the form of morphological and mass measurements. The hatchlings were measured prior to the start of the experiment, to establish a baseline, starting size. As previously stated, the measurements were taken every four weeks for a nine (9) month period. In order to take the measurements, all turtles were removed from the pond and placed in temporary containers large enough to hold all turtles from the bin and included water to prevent desiccation. Because the turtles were fed different diets, it was important to keep each bin separate. Each turtle was identified by its individual number using the notches in the marginal scutes (Fig. 5). All morphological measurements were recorded to the nearest tenth of a millimeter (0.1 mm) using analog calipers, and mass was measured to the nearest gram using a digital gram scale. The morphological measurements were recorded for each surviving individual. The dataset included all measurements of all morphological characters for each turtle that was alive at the time measurements were taken.

Each turtle was measured for six (6) morphological characters at each measuring period. Straight-line measurements were taken using analog calipers for the following characters: plastron length (PL), plastron width (PW), carapace length (CL), carapace width (CW), and dorso-ventral depth (Depth). A digital gram scale was used to measure mass (M). Each measurement was recorded for future analyses.

Plastron length (PL) was used as the determining character for body size and growth. Growth was analyzed by repeated measures analysis of variance (ANOVA) using the class variables Diet (High, Medium or Low) and Measure Period (1 – 9).

Statistical Analyses

Statistical analyses were performed with S-Plus® for Windows version 6.1. We determined the effect of variable protein diet on growth as seen by morphological characters and mass with repeated measures analysis of variance. The data met requirements for normality (Appendix IV, a) and homoscedasticity (homogeneity of variance) (Appendix IV, b), so no transformations were performed.

RESULTS OF STUDY

A. GROWTH (DURING EXPOSURE TO VARIABLE DIETS)

In total, 185 turtles survived the 9-month variable protein portion of the experiment. Observations from these turtles resulted in nearly 22,800 measurements for the six characters. We anticipated that the turtles would grow over the course of the experiment, so it was not unexpected that the final measurements of the surviving turtles were all larger than the initial measurements. All measurements noted the same upward trend over the course of the experiment (Appendix II, a-e).

Not surprisingly, the turtles fed the high (42%) protein diet grew more quickly and showed the greatest increase in size over the course of the initial 9-month feeding period, as measured by all morphological and mass measurements. A repeated measures analysis of variance (ANOVA) (S-Plus, 2002) was performed on the data for each morphological character to determine the effect the different protein levels have on growth in the "hatchling to juvenile" stage of *G. nigrinoda*. This test allowed us to determine if there were differences among the protein levels (high, medium, or low). All measurement characters had the same starting measurement. For all six measurements, growth rates were significantly different across all three protein levels. Turtles on the high protein diet grew faster than the turtles in the medium and low protein diets as evidenced by the greater morphological and mass measurements (Appendix II, a-e). For each morphological or mass measurement, the high protein diet was significantly

greater than the medium or low diets by month 4, and all three diets were significantly different by month 7, (Appendix II, a-e). All measurements indicated the same trend throughout the experiment.

Because all measurements were related to size and expected to respond to time, there was likely to be high correlation between each morphological character. A Pearson's product-moment correlate test was performed using S-Plus® (2002) on each pair-wise grouping of morphological characters. A scatter plot was generated for all pair-wise comparisons for all measurement characters (Fig. 7). For brevity and because those correlation values were very high (0.9840 to 0.9980) it is unnecessary to report all findings for all 6 morphological measurements. As previously stated, all morphological characters and mass indicated the same trend over time. Therefore only the results for one morphological character, plastron length (PL), are given (Fig. 8). This character is of greatest relevance to field studies and subsequent captive works. This character was used for statistical analyses as well as reporting purposes.

B. Growth (Post-Variable Protein Portion of the Experiment)

After 9 months of the uniform diet portion of the experiment, the juvenile turtles were again measured for the six (6) morphological characters, and again, only plastron length is reported. The assumptions for normality and homoscedasticity (Appendix IV, a and b) were not violated, so no transformations were required. After nine months on the uniform diet, there were no differences in growth rates in the turtles, regardless of previous diet treatment ($P = 0.5$) (Fig. 9).

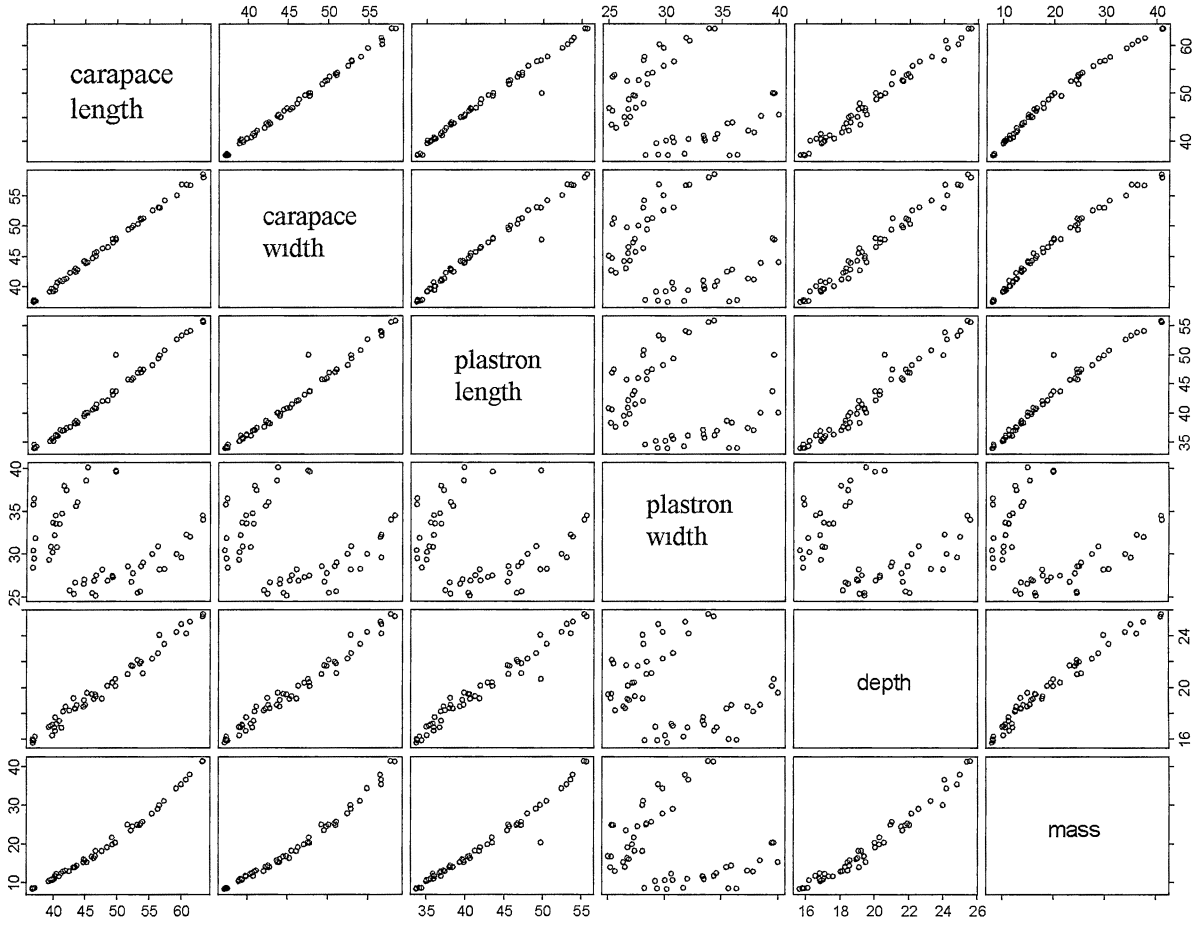


FIGURE 7 Scatter plot of all pair-wise comparisons of the six measurement characters used in this evaluation of variable dietary protein on juvenile *Graptemys nigrinoda*

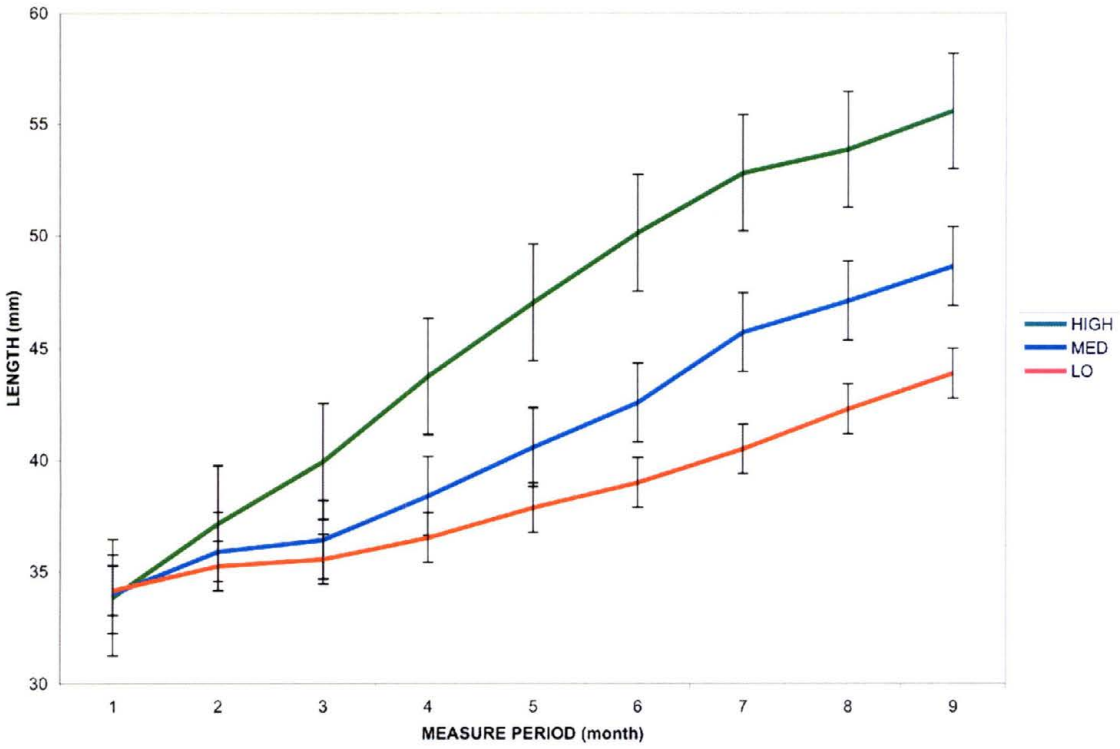


FIGURE 8. The average plastron length for each of the three protein levels (High, Med, and Low) for the duration of the experiment (9 months.) Plastron length was statistically the same across all three diets when the experiment began. By measure period (month) 4, plastron length of the turtles in the high protein diet was significantly different from the other two diets. By measure period (month) 6, plastron length of the turtles in each diet was significantly different from the plastron length of the turtles in the other diets.

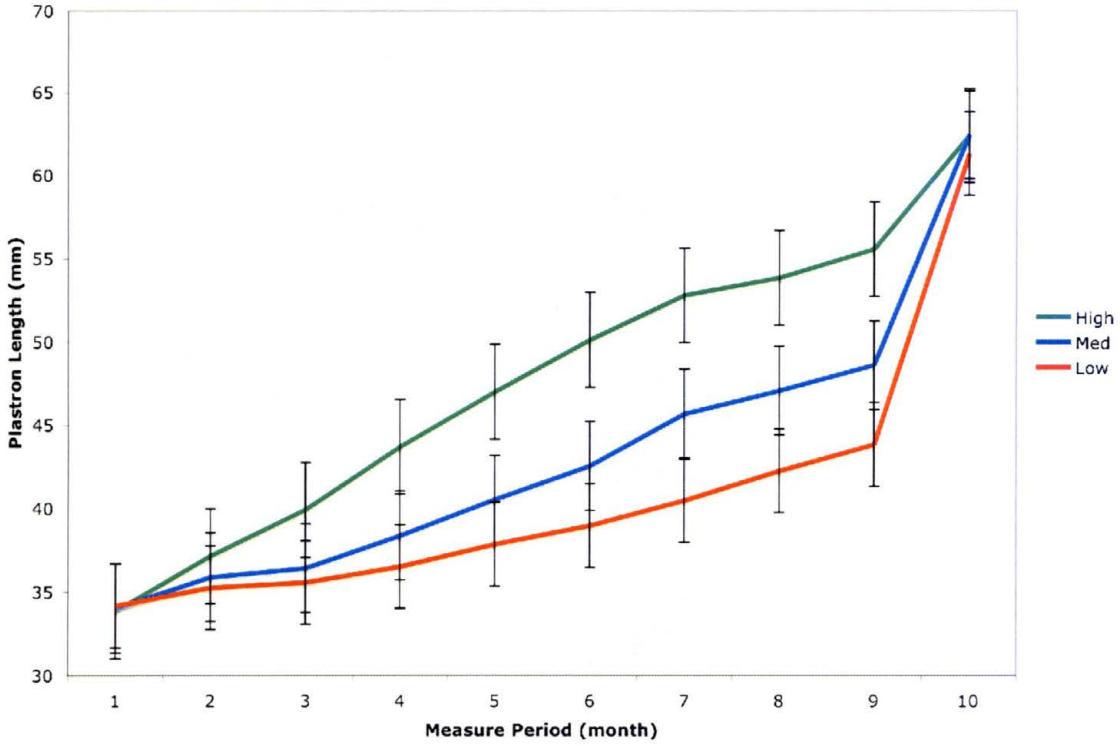


FIGURE 9. Average plastron length over time for *Graptemys nigrinoda* for the current study. This graph includes measure period 10, the final measurement. The final measurement is statistically equal across add protein diets, e.g. there is no difference in growth based on protein level after 9 months of uniform diet.

C. GROWTH OF *GRAPTEMYS NIGRINODA* IN COMPARISON TO OTHER *GRAPTEMYS* SPECIES USING P. LINDEMAN'S PROJECTED GROWTH CURVES.

Growth curve estimates for four (4) *Graptemys* species, *G. ouachitensis*, *G ernsti*, *G. pseudogeographica*, and *G. cageli*, have been identified (Lindeman, 1999) using plastron lengths of known *Graptemys*. We used these Lindeman growth curves as comparisons to the *G. nigrinoda* from this study. Lindeman used both males and females when available, or only one gender when both were not available; therefore there are sometimes two curves for his data. The *G. nigrinoda* from the present study were used randomly, and the turtles were too immature to identify by gender; therefore there are no differences by gender. The *G. nigrinoda* in each protein treatment from this study were compared to each of the *Graptemys* species from the Lindeman (1999) analyses. This approach allowed recognition of any similarities across species within the same genus for the same age (0-0.75 years) (Fig. 10).

I. *Graptemys nigrinoda* compared to *G. ouachitensis*

There is a significant difference ($df = 4$, $P<0.0001$) between the *G. nigrinoda* growth curve and the projected curve of *G. ouachitensis* (Appendix I, g). The *G. nigrinoda* data from this study were above the *G. ouachitensis* data, suggesting faster and greater growth during this portion of the life stage. After performing the Tukey's post hoc test, the differences between groups are apparent (Appendix III, a). The High and Medium *G. nigrinoda* groups are statistically different from both male and female *G ouachitensis* projected growth curves during this portion of the growing period (age 0-0.75 years); however, *G nigrinoda* Low is not different from *G. ouachitensis*.

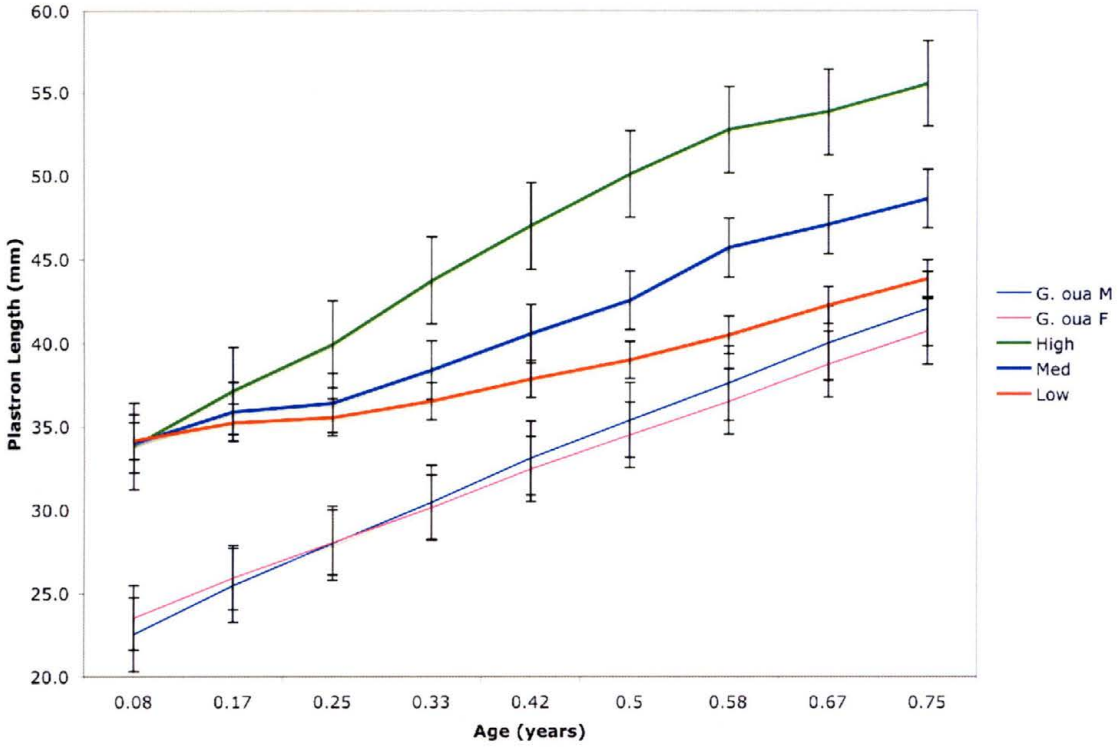


FIGURE 10. *Graptemys nigrinoda* data from this study compared to Lindeman's projected growth curves for *G. ouachitensis.* The variables are *G. nigrinoda* – High, Med, and Low and *G. ouachitensis* – Male and Female. All growth curves represent the same duration (0 - 0.75 years).

II. *Graptemys nigrinoda* compared to *G. cageli*

There is a significant difference (df = 4, P=0.036) (Appendix I, h) among the *G. nigrinoda* growth curves and the projected curve of *G. cageli* (Appendix II, f). The *G. nigrinoda* from this study appeared very similar to the *G. cageli* projected growth curve (Fig. 10). In fact, after performing the Tukey's post hoc test, the only curves that were significantly different in the *G. nigrinoda* and the *G. cageli* over this life stage were the *G. nigrinoda* High and *G. nigrinoda* Low curves (Appendix III, b).

III. *Graptemys nigrinoda* compared to *G. pseudogeographica*

The ANOVA from the comparison of growth curves for *G. nigrinoda* and *G. pseudogeographica* showed a significant difference (df = 3, P=0.031) (Appendix I, i). The growth curves of the *G. nigrinoda* data from this study were similar to the growth curve of the projected growth curve from Lindeman (Appendix II, g). After performing the Tukey's post hoc test, (Appendix III, c) only two curves remained significantly different, the *G. nigrinoda* High from this study and the projected growth curve for the *G. pseudogeographica* Female.

IV. *Graptemys nigrinoda* compared to *G. versa*

Growth curves for *G. nigrinoda* from this study and the projected *G. versa* growth curves were significantly different (df = 3, P<0.0001) (Appendix I, j). The *G nigrinoda* curves do not cross the *G. versa* curves and appear to have different slopes (Appendix II, h). For this comparison, the Tukey's post hoc test was useful in finding the differences in the slopes the ANOVA indicated (Appendix III, d). After performing the post hoc test, only the *G. nigrinoda* High from this study and the *G. nigrinoda* Low from this study were statistically different. All other comparisons were not significantly different.

Overall, *Graptemys* growth curves were consistent. The different species of *Graptemys* used by Lindeman (1999) for these analyses were comparable to the *G nigrinoda* from this study.

One thing to keep in mind regarding the projected growth curves from Lindeman's data is that the *G. cageli* and *G. versa* data are comprised of only one gender (male and female, respectively) to determine the growth curves, while the *G. nigrinoda* data and the two other Lindeman curves use both genders. This is not likely to be of any consequence at this life stage because hatchling turtles grow at the same rate, regardless of gender (Ernst, *et al.*, 1972.)

D. MORTALITY RATES

Mortality was high in the experiment (Fig. 11), and many juveniles died during the course of the experiment. This was a predictable response for working with organisms with Type III survivorship curves (Fig. 3). Mortality was different in at least two of the treatments as indicated by the significant p- value (P = 0.0002) (Appendix I). Because the ANOVA does not indicate which treatments are different, merely that there is a difference in at least two of them, a Tukey's Multiple Comparison post hoc test was performed. The results from the post hoc test indicated that turtles in the each diet treatment suffered mortalities at different rates than turtles in the other treatments (Appendix III, e). While we expected high mortality for the juveniles in the experiment, we did not expect variable mortality across the protein diets. The juveniles in the Low protein bin suffered the highest mortality, followed by the medium protein, and finally the high protein. There is a significant difference in the mortality rate (P = 0.0002)

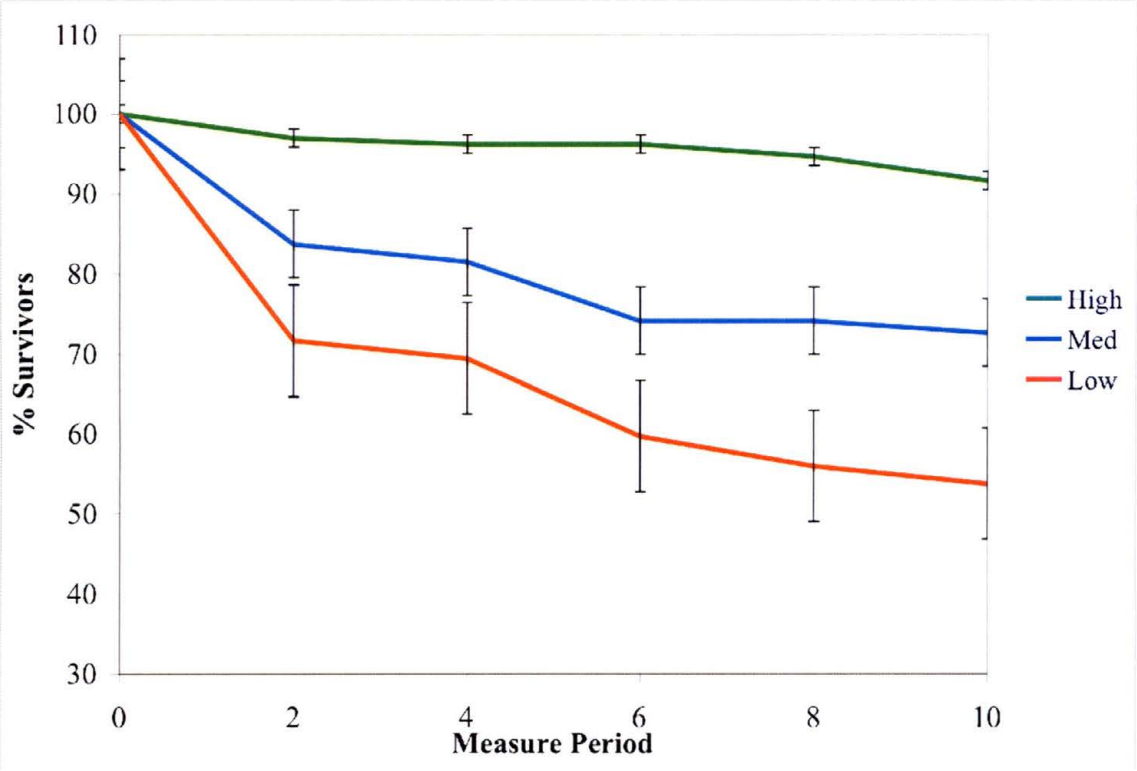


FIGURE 11. Mortality of *Graptemys nigrinoda* throughout the evaluation of dietary protein effects using three protein diets. Prior to measure period 10 (the second 9-month phase of the experiment), both cohorts are represented; for measure period 10, only data from the first cohort is represented. Mortality was significantly different across the different protein levels (P = 0.0002).

of the turtles across each protein treatment. The statistical evidence confirms the visual impression from Figure 11; juveniles reared on the low protein diets suffered greater mortalities than juveniles reared on the medium protein, and juveniles reared on the medium protein diets suffer greater losses than juveniles reared on the high protein diets. The first cohort experienced higher mortality in comparison to the second cohort for reasons that are unknown.

DISCUSSION

GROWTH (DURING EXPOSURE TO THE VARIABLE DIETS)

The results of this study are clearly applicable to captive propagation. A goal of both captive breeding and head starting is to release animals with improved survivorship over that of the wild population (Heppell, *et al.,* 1996). One way to help ensure improved survival is by reducing the threat of juvenile mortality and thereby changing the slope of the mortality curve of these Type III organisms. A species' chances of prolonged recovery are better if more juveniles reach adulthood.

The turtles grew over the course of the experiment, as expected. The hatchling and juvenile stages of a turtle's life are characterized by more rapid growth rates than any other life stage (Ernst, 1972). In this experiment, growth was measured by six measurements, and these morphological and mass measurements are common for cataloging turtles (Cagle, 1948; Ernst et al, 1972; Alho, 1985; Frazer, 1986; Avery 1987.)

For this life stage, I determined that plastron length was one of the most useful measurements for categorizing growth. While this and other morphological measurements may be indicators of growth, for this study, plastron length was the most accurate and efficient. Turtles are likely to suffer injuries on their carapaces, such as accidents with recreational vehicles (boats) or interaction with a predator. These injuries can damage or remove marginal scutes making carapacial measurements inaccurate and underestimated.

While not a consideration for this experiment, plastron width and depth often asymptote during later life stages. There is a point at which these measurements do not grow at the same rate as other morphological growth indicators, such as plastron length. Dorso-ventral depth can be a valid measure of growth, especially if trying to differentiate based on gender, because females will have a larger depth measurement as they mature. Females are larger than males due to increased egg capacity inside the body cavity. Mass is not always an accurate measure of size because during times when resources are scarce, a turtle's size may not be truly indicated by mass.

Growth (Post-Variable Protein Portion of the Experiment)

The hatchlings subjected to the high protein level grew faster during the initial 9-month portion of the experiment (Fig. 8). The turtles fed the high protein diet did not retain an increased growth rate after the variable diet portion of the experiment was concluded. Figure 9 shows the final measurement, and this value is not statistically different for the three treatments. Any advantage the high protein group had during the variable portion of the experiment was lost. Possibly, the low and medium protein diets had a period of rapid growth during this time and the growth advantage was reduced by this phenomenon. Once removed from the variable portion of the experiment and all fed a uniform, medium (32 % Protein) diet, there was no "growth advantage" or "growth disadvantage" of any one diet over any other. There are several explanations. First, the hatchlings were not subjected to the variable protein portion of the experiment long enough to have lasting effects from the other protein levels. This implication is crucial when considering this technique as a conservation tool. It is not enough to know that the

turtles fed the high protein diet grew at a significantly faster or greater rate; knowing how long the turtles require this diet in order to maintain this projected growth is equally important. While Gibbons (1967) reported that growth differences could be attributable to food quality, the duration of feeding on quality food required for a sustained "growth advantage" should be investigated.

Second, these juveniles may have reached the portion of their life histories where sustainable, rapid growth is no longer likely. This implication relies on other factors as well, for instance, that these juveniles have reached or are nearing sexual maturity. It is unlikely that the juveniles were sexually mature at this point because they were < 4 years in age (males) and 7 years of age (females), which are common ages to reach sexual maturity in similar taxa, (Frazer, et al., 1990, Cagle 1948), and/or the average plastron length was < 9 cm (Cagle, 1948). Lindeman showed that *Graptemys* are likely to require even more time (up to 7 years for male and 11 + years for females) to reach maturity (1999). However, at the culmination of the 9-month variable-protein portion of the study, most of the juveniles in the high and medium protein levels were gender-identifiable, possibly indicating that they were closer to maturation.

Mortality Rates

Because turtles are examples of Type III survivorship organisms (Fig. 3) (Wilbur, 1975; Frazer, 1992; Heppell, *et al.*, 1996.) with high reproductive output marked by few long-term survivors, we did anticipate high mortality for the hatchlings. We also expected most of this mortality early in the study. We observed more deaths early (months 1-3) in the study, but high mortality was not restricted to this time period. While

we expected this taxon to experience high mortality, we would not have predicted as high a difference to manifest consequent of diet variation. Another interesting finding of this experiment was that the turtles fed the lower protein diet experienced higher mortality rates over the other two diets (Fig. 11). This is especially important since mortality has an obvious impact on how juvenile turtles raised on pelleted diets should be managed.

Mortality rates are of serious consequence for management of organisms with low juvenile survivorship. Little information is available about true mortality rates in the wild because juveniles represent a very small percentage of wild turtle populations, and demographic data are lacking for so many non-marine turtles (Reed & Gibbons, letter to US Dept of the Interior and USFWS, 2001). Juvenile survival is low in captivity, and without empirical data, we can tentatively assume survival is at least as low in wild populations. Any effort to reduce this mortality may result in increased survival rates for juveniles and help shift the mortality curve from Type III more towards Type II or Type I. Captive breeding and head starting have the ability to increase survivorship by reducing predators, increasing availability of resources, and reducing competition among juveniles. These effects may result in animals with a better than average chance of reaching adulthood.

GROWTH OF *GRAPTEMYS NIGRINODA* IN COMPARISON TO OTHER *GRAPTEMYS* SPECIES USING P. LINDEMAN'S PROJECTED GROWTH CURVES

Based on multiple lines of evidence (Lamb, *et al.*, 1994; Shaffer, *et al.*, 1997; Stephens & Wiens, 2003) *Graptemys* species are all closely related. However, there is discrepancy as to the precise phylogenetic relationships among these taxa, as seen by the

numerous topologies just mentioned. In one publication alone, *G nigrinoda* was found to be most closely related to three different *Graptemys* species, *G oculifera*, *G. versa*, and *G cageli* (Stephens & Wiens, 2003). This result occurred because three different gene regions were used to determine phylogenies, and these regions presumably diverged at different rates. It is safe to say phylogenetic relationships are ambiguous. The comparisons across *Graptemys nigrinoda* in this experiment and projected growth curves of four other *Graptemys spp* (Lindeman, 1999) show that these taxa exhibit similar growth rates (Fig. 10 and Appendix II, f and h). If *G. nigrinoda* respond in a similar way to other closely related *Graptemys* then results may be extrapolated to other closely related taxa, and might be applicable beyond the genus.

Implications of Recommendations for Use

There are extensive studies for other taxa that show there is a relationship between growth and maturity (Gibbons, *et al.*, 1980; Frazer, *et al.*, 1990). These organisms require a long time to mature and have low reproductive success. If an increase in protein in the diet allows for increased growth and faster maturation, this phenomenon could decrease the time required to reach sexual maturity and possibly result in the opportunity to reproduce more often. By reproducing more often, it might be possible to increase reproductive output and consequent recruitment in the population.

Because the measurements used in this study are standards of growth, they have the potential to be highly correlated; any measurements that measure growth are likely to change in the same proportion as the growth of the organism being measured. Therefore, it is important to recognize the high correlation between these values, and fewer

measurements (or even one) could have sufficed. Significant researcher time could have been saved by just using the plastron length as a measure of growth for juvenile turtles. This is especially important, not for an experiment of this size, but rather for one where thousands of turtles require cataloging.

Turtles fed on high protein diets for the duration of their captivity may be bigger than turtles fed lower protein levels, and this data show this to be true at least while the juvenile turtles were exposed to the different treatments. This effect may or may not lead to more successful adults, and the larger turtles may not remain larger over time. One effect is obvious, however. Those turtles fed high protein diets in captivity are less likely to die.

Implications of this research are pragmatic and can be implemented easily for other captive propagation programs. This information could lead to different responses in captive rearing and managing of captive raised populations or in supplementing wild populations.

Viable Solutions for Endangered Species?

The debate as to whether captive propagation and head starting are viable solutions rages on (Frazer, 1992; Tear, *et al.*, 1995; Heppell, *et al.*, 1996; Snyder, *et al.*, 1996; Balmford, *et al.*, 1996; Gippoliti & Carpaneto, 1997; Hunt, 2003.) Many believe that as management tools, head starting and captive propagation can only be successful if they limit factors such as behavioral changes and domestication (Snyder, *et al.*, 1996) or increase population growth significantly (Heppell, et al., 1996) and certainly will not be successful if captive propagation is not used in conjunction with other conservation

efforts, such as habitat repair (Lydeard and Mayden, 1995; Snyder, *et al.*, 1996; Gippoliti and Carpaneto, 1997.)

The reality is that captive propagation has worked. It worked for animals such as the American Alligator (*Alligator mississipiensis*) (Gigon, *et al.*, 2000) and continues to be a conservation tool for marine turtles (*Caretta caretta*, *Chelonia mydas*) (Huff, 1989.) The American Alligator remains one of a very short list of animals removed from the Endangered Species List. This can only be achieved by proving beyond a doubt that a species has recovered. As more information becomes available as to the successes and techniques for captive propagation for other reptiles including turtles, we will continue to see the benefits and further applications of this aggressive conservation technique.

Captive propagation may not be the best answer. It has limits and should not be attempted without other conservation techniques in place (Heppell and Crowder, 1996). However, unless we can reverse the habitat loss and destruction affecting habitat, it falls to us as stewards of the environment, and its inhabitants, to protect what we have left.

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APPENDIX I

Results from the repeated measures analysis of variance (ANOVA). Results were calculated using S-Plus statistical software and are given as tables with response variable indicated. Unless otherwise specified, the analyses are for the first 9-month phase of the experiment. Data comparing *G. nigrinoda* for this study and *Graptemys sp.* from Lindeman (1999) are also provided. All ANOVA factors are provided along with relevant F statistics and P-values.

Source	df Num	df Den	F-value	P-value
Replicate	1	26	56923.22	<0.001
Meas Pd	8	26	124.73	<0.001
Diet	2	26	217.61	<0.001
Meas Pd x Diet	16	26	8.75	<0.001

Note: Variables: "Replicate" = Replicate of Diet Level (n=2), "Meas Pd" = Measure Period (1-9), and "diet" = Diet (High, Medium, and Low).

a. ANOVA results for the morphological character plastron length for *Graptemys nigrinoda* in the evaluation of dietary protein effects on juvenile turtles. All P-values are significant and indicate differences.

Source	df Num	df Den	F-value	P-value
Replicate	1	26	23469.9	<0.001
Meas Pd	8	26	195.13	<0.001
Diet	2	26	292.54	<0.001
Meas Pd x Diet	16	26	11.56	<0.001

Note Variables: "Replicate" = Replicate of Diet Level (n=2), "Meas Pd" = Measure Period (1-9), and "diet" = Diet (High, Medium, and Low)

b. The ANOVA results for the morphological character plastron width for *Graptemys nigrinoda* in the evaluation of dietary protein effects on juvenile turtles. All P-values are significant and indicate differences.

Source	df Num	df Den	F-value	P-value
Replicate	1	26	34709.59	<0.001
Meas Pd	8	26	474.37	<0.001
Diet	2	26	656.05	<0.001
Meas Pd x Diet	16	26	25.64	<0.001

Note Variables "Replicate" = Replicate of Diet Level (n=2), "Meas Pd" = Measure Period (1-9), and "diet" = Diet (High, Medium, and Low)

c. ANOVA results for the morphological character carapace length for *Graptemys nigrinoda* in the evaluation of dietary protein effects on juvenile turtles. All P-values are significant and indicate differences.

Source	df Num	df Den	F-value	P-value
Replicate	1	26	26155.79	<0.001
Meas Pd	8	26	481.43	<0.001
Diet	2	26	624.54	<0.001
Meas Pd x Diet	16	26	23.12	<0.001

Note: Variables: "Replicate" = Replicate of Diet Level (n=2), "Meas Pd" = Measure Period (1-9), and "diet" = Diet (High, Medium, and Low)

d. ANOVA results for the morphological character carapace width for *Graptemys nigrinoda* in the evaluation of dietary protein effects on juvenile turtles. All P-values are significant and indicate differences

Source	df Num	df Den	F-value	P-value
Replicate	1	26	48560.25	<0.001
Meas Pd	8	26	150.15	<0.001
Diet	2	26	253.46	<0.001
Meas Pd x Diet	16	26	9.07	<0.001

Note: Variables: "Replicate" = Replicate of Diet Level (n=2), "Meas Pd" = Measure Period (1-9), and "diet" = Diet (High, Medium, and Low)

e. ANOVA results for the dorso-ventral depth for *Graptemys nigrinoda* in the evaluation of dietary protein effects on juvenils turtles. All P-values are significant and indicate differences.

Source	df Num	df Den	F-value	P-value
Replicate	1	26	2630.579	<0.001
Meas Pd	8	26	215.632	<0.001
Diet	2	26	349.789	<0.001
Meas Pd x Diet	16	26	17.724	<0.001

Note. Variables "Replicate" = Replicate of Diet Level (n=2), "Meas Pd" = Measure Period (1-9), and "diet" = Diet (High, Medium, and Low).

f. ANOVA results for the mass measurement for *Graptemys nigrinoda* in the evaluation of dietary protein effects on juvenile turtles. All P-values are significant and indicate differences

Source	Df	Sum of Sq	Mean Sq	F Value	P-Value
Turtle	4	1202.434	300.6084	8.437535	0.0000492671
Residuals	40	1425.100	35.6275		

Note: Variables: "Turtle" = *Graptemys nigrinoda* High, Med, and Low; *G ouachitensis* Male and Female "Residuals" = Error

g. ANOVA results from growth curves comparing *Graptemys nigrinoda* in this study of the evaluation of variable diet on juvenile turtles and the projected growth curves of *G ouachitensis (*Lindeman, 1999). The P-value indicates differences.

Source	Df	Sum of Sq	Mean Sq	F Value	P-Value
Turtle	4	351.0148	87.7537	2.943639	0.03583179
Residuals	31	924.1503	29.8113		

Note· Varıables. "Turtle" = *Graptemys nıgrınoda* Hıgh, Med, and Low; *G cageli* "Resıduals" = Error

h. ANOVA results from growth curves comparing *Graptemys nigrinoda* in this study of the evaluation of variable diet on juvenile turtles and the projected growth curves of *G. cageli (*Lindeman, 1999).

Source	Df	Sum of Sq	Mean Sq	F Value	P-Value
Turtle	4	470.821	117.7052	2.957813	0.03125029
Residuals	40	1591.787	39.7947		

Note Variables "Turtle" = *Graptemys nigrinoda* High, Med, and Low, *G pseudogeographica* "Residuals" = Error

i. ANOVA results from growth curves comparing *Graptemys nigrinoda* in this study of the evaluation of variable diet on juvenile turtles and the projected growth curves of *G. pseudogeographica (*Lindeman, 1999). The P-value indicates differences.

Source	Df	Sum of Sq	Mean Sq	F Value	P-Value
Turtle	3	1035.224	345.0747	10.80222	0.00004644802
Residuals	32	1022.234	31.9448		

Note. Variables: "Turtle" = *Graptemys nigrinoda* High, Med, and Low and *G versa* "Residuals" = Error.

j. ANOVA results from growth curves comparing *Graptemys nigrinoda* in this study of the evaluation of variable diet on juvenile turtles and the projected growth curves of *G. versa* (Lindeman, 1999). The P-value indicates differences.

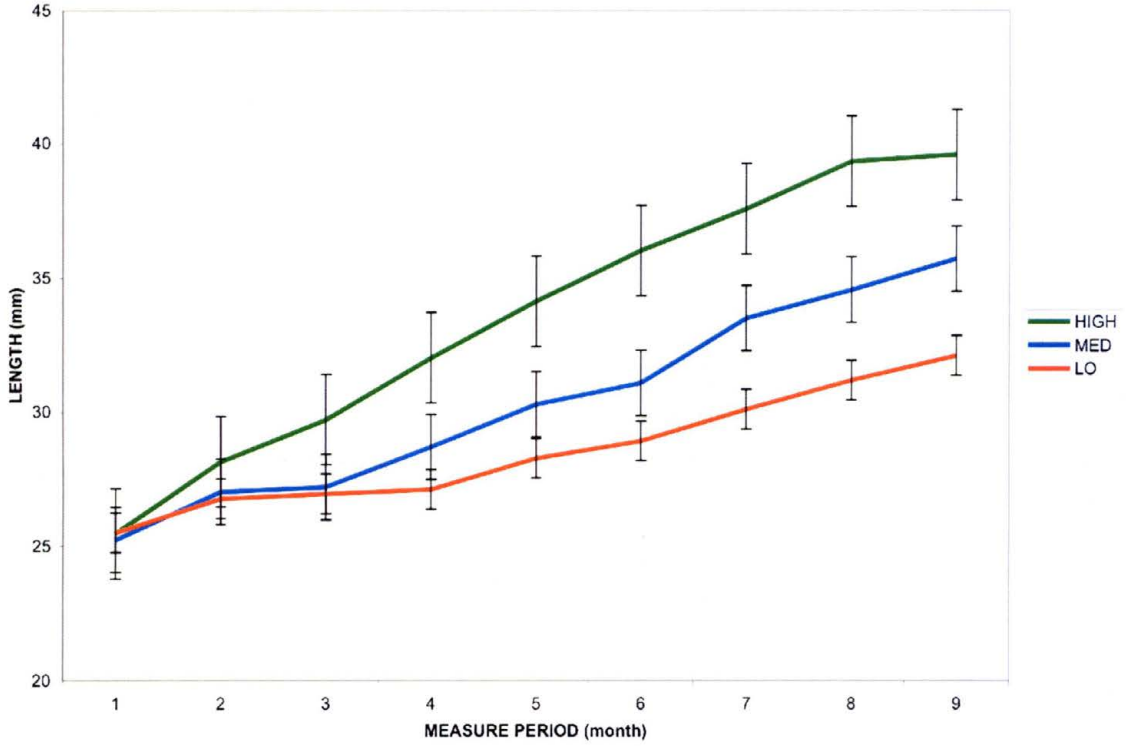
Source	Df	Sum of Sq	Mean Sq	F Value	P Value
Measure Period	5	1483.542	296.708	5.45487	0.01116357
Diet	2	2277.763	1138.882	20.93789	0.00026618
Residuals	10	543.933	54.393		

Note: Variables: "Measure Period" = Measure Period (1-10), Diet = Diet (High, Medium, Low)

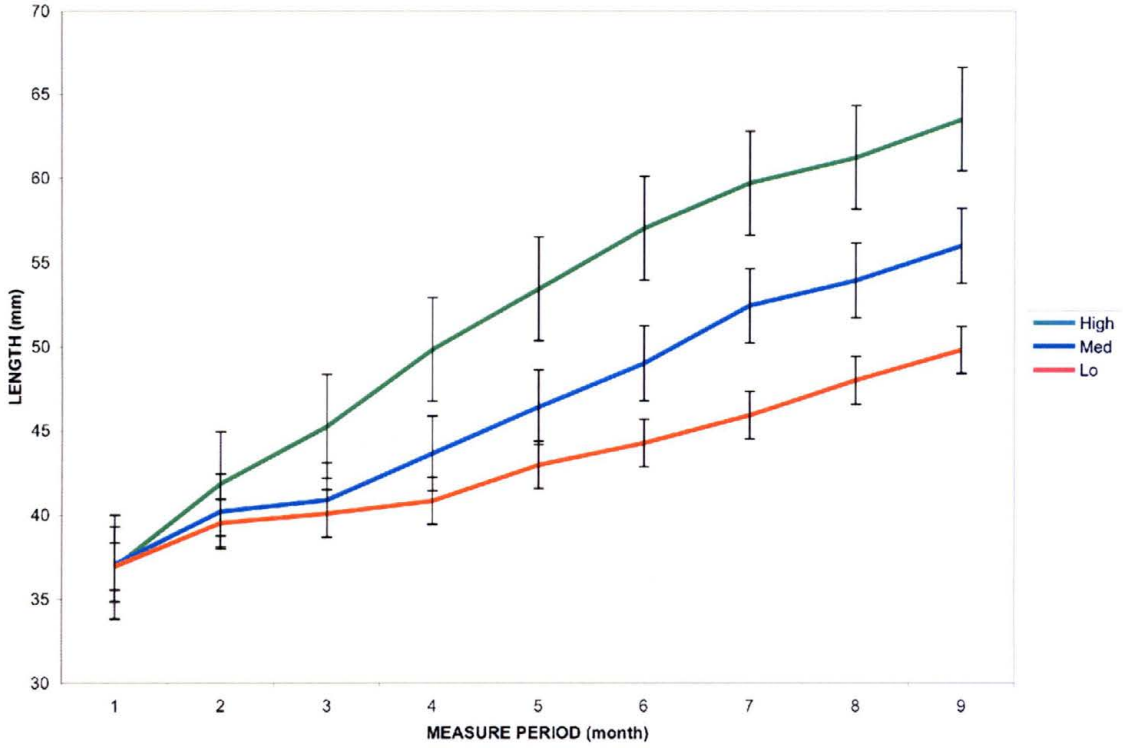
k. ANOVA results for mortality rates across the three protein treatments from the evaluation of variable protein diet on juvenile *Graptemys nigrinoda*. The P-value indicates differences in the mortality rates.

APPENDIX II

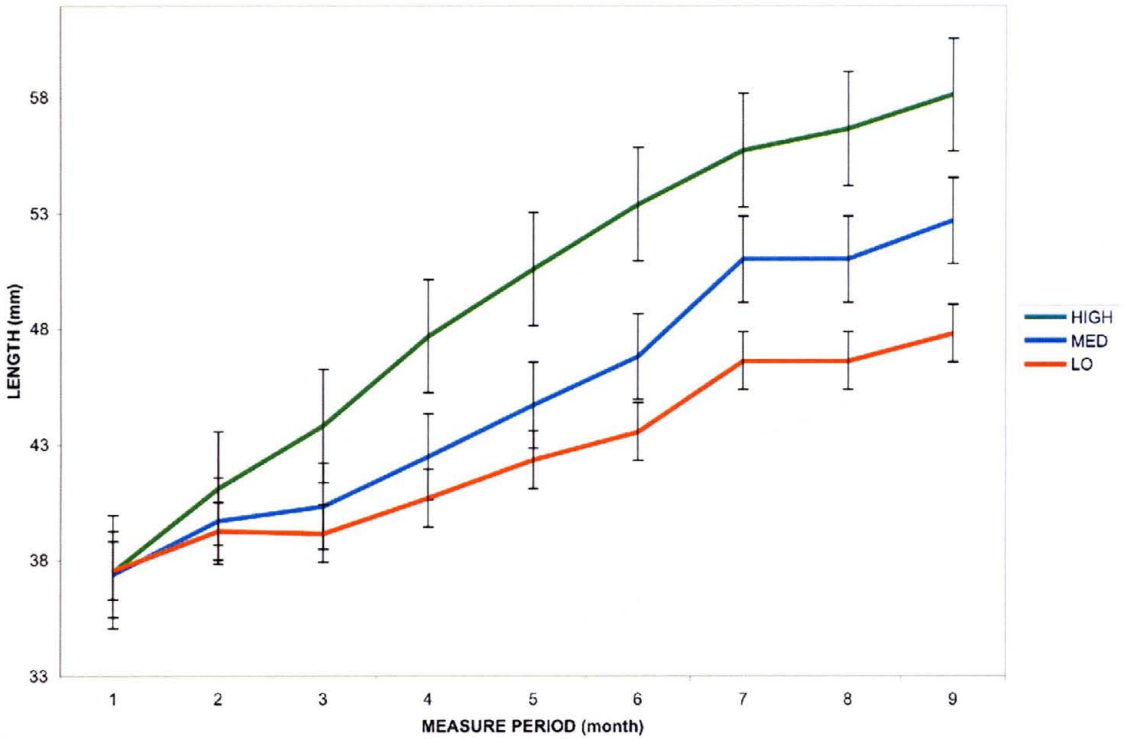
Growth curves for the morphological characters and mass characters used in the evaluation of variable protein diet on juvenile *Graptemys nigrinoda*. Unless otherwise indicated, the growth curve represents the first 9-month phase of the experiment (exposure to variable protein levels).



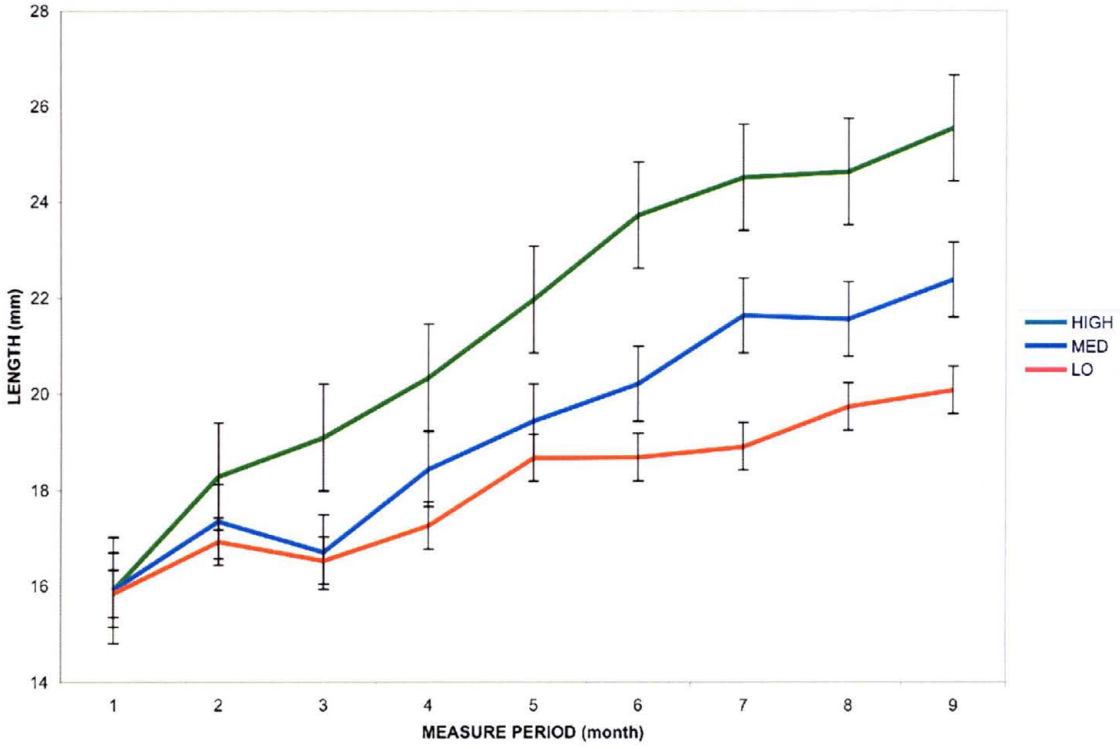
a. Growth curves of juvenile *Graptemys nigrinoda* for mean plastron width for each of the three protein levels (High, Med, and Low) over the first 9-month phase of the effects of dietary protein on juvenile turtles. Plastron width was significantly greater in the high protein diet from the other two diets.



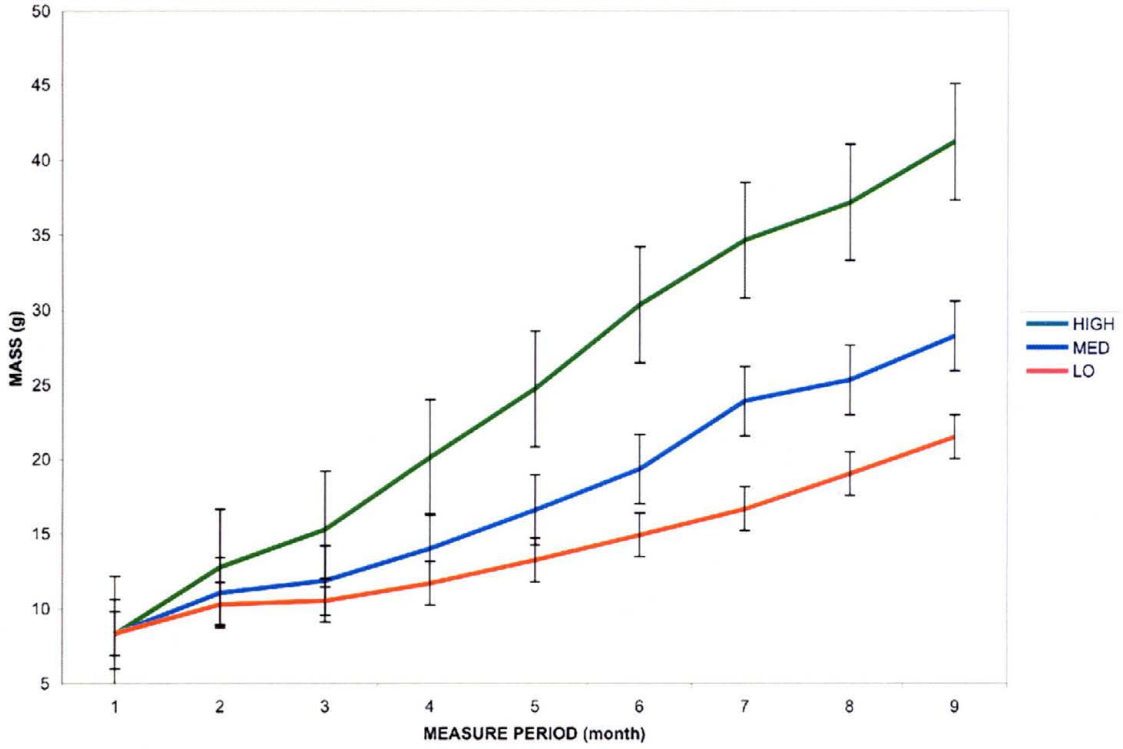
b. Growth curves of juvenile *Graptemys nigrinoda* for mean carapace length for each of the three protein levels (High, Med, and Low) over the first 9-month phase of the effects of dietary protein on juvenile turtles. Carapace length was significantly greater in the high protein diet from the other two diets.



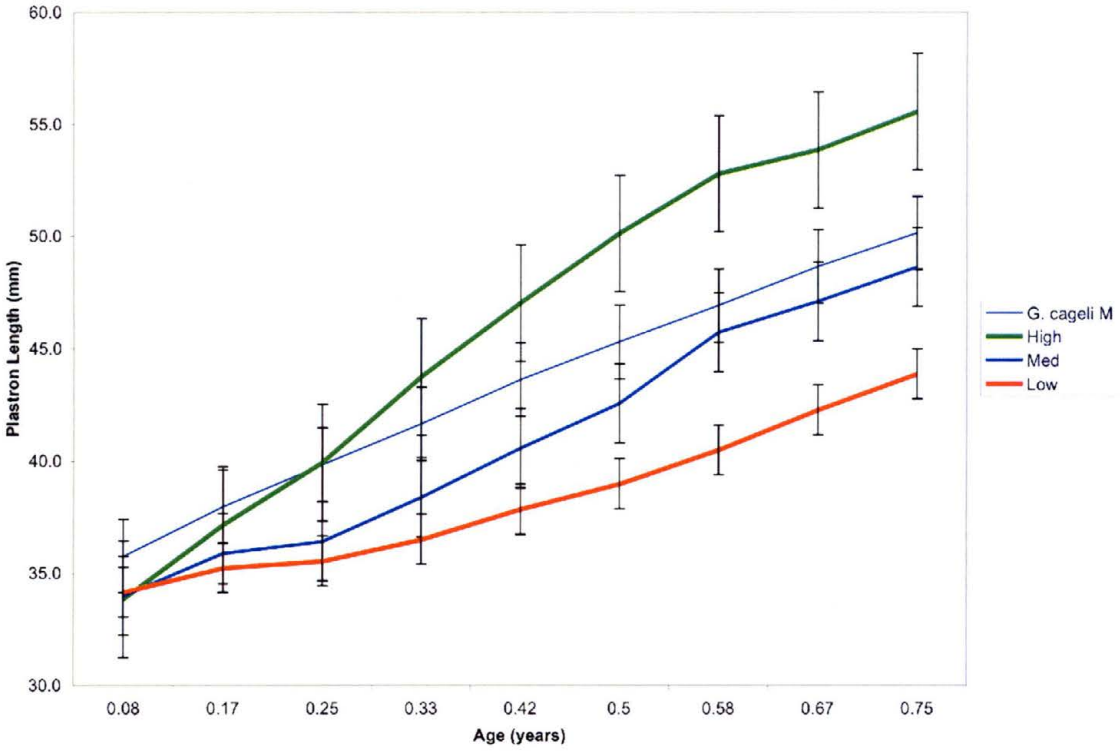
c. Growth curves of juvenile *Graptemys nigrinoda* for mean carapace width for each of the three protein levels (High, Med, and Low) over the first 9-month phase of the effects of dietary protein on juvenile turtles. Carapace width was significantly greater in the high protein diet from the other two diets.



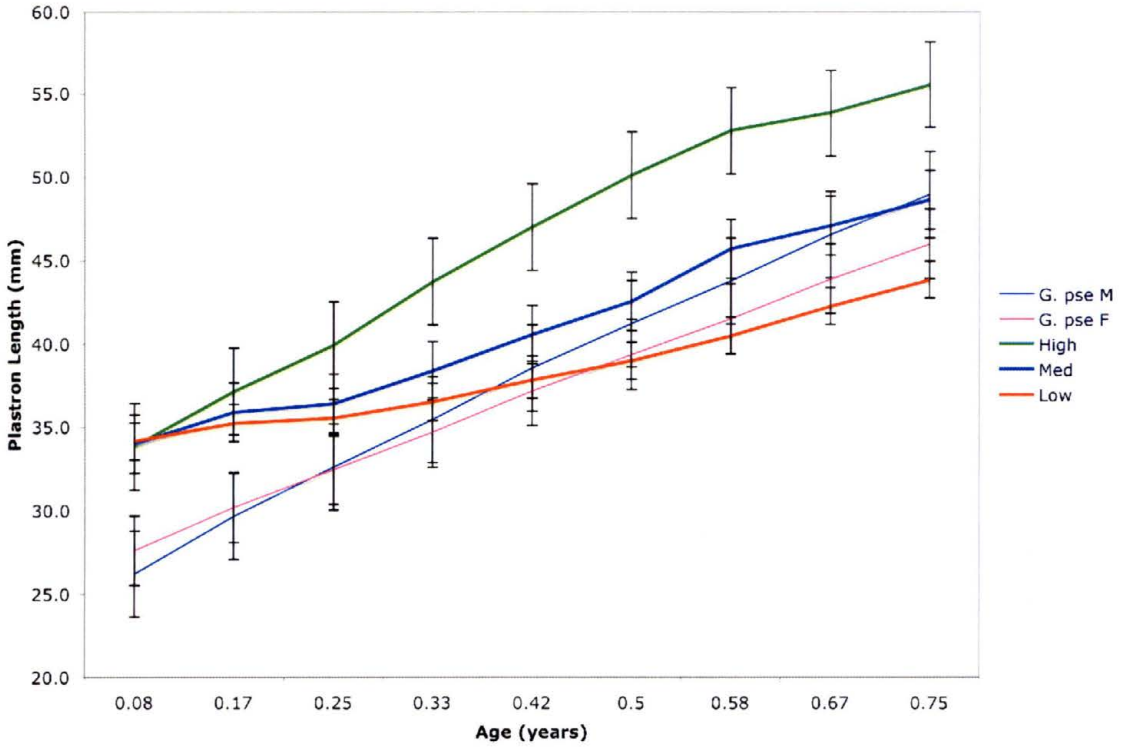
d. Growth curves of juvenile *Graptemys nigrinoda* for mean dorso-ventral depth for each of the three protein levels (High, Med, and Low) over the first 9-month phase of the effects of dietary protein on juvenile turtles. Dorso-ventral depth was significantly greater in the high protein diet from the other two diets.



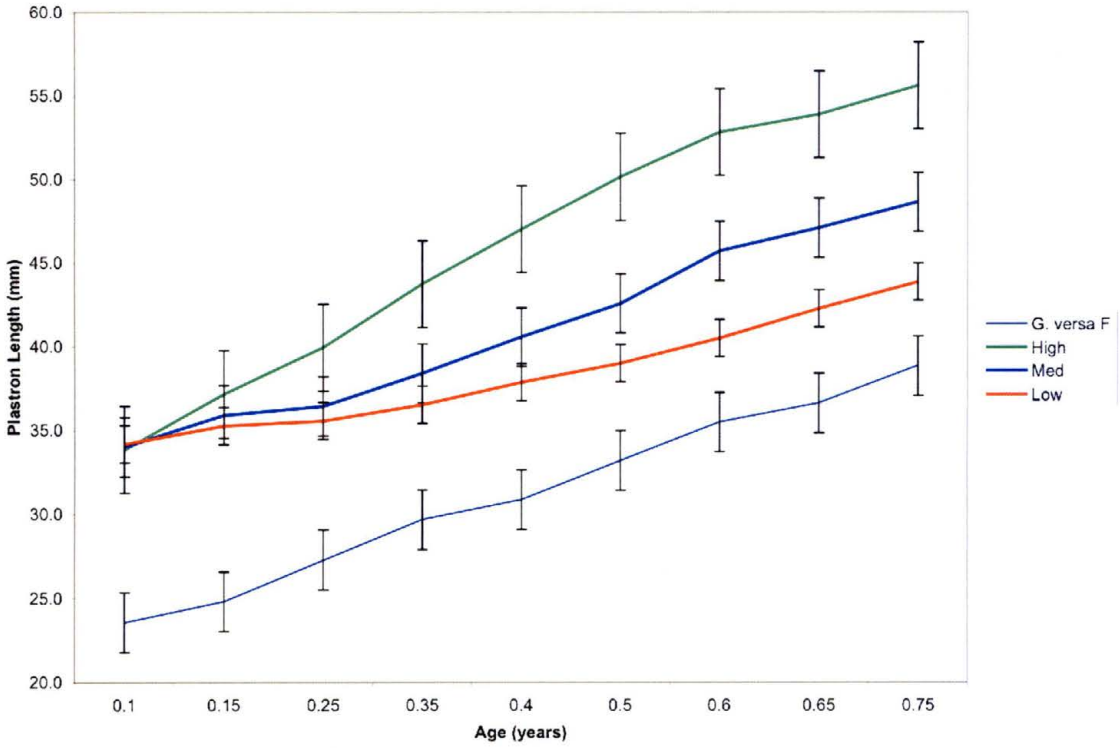
e. Growth curves of juvenile *Graptemys nigrinoda* for the mean mass for each of the three protein levels (High, Med, and Low) over the first 9-month phase of the effects of dietary protein on juvenile turtles. Mass was significantly greater in the high protein diet from the other two diets.



f. Growth curves for the juvenile *Graptemys nigrinoda* data from this evaluation of dietary protein compared to projected growth curves for *G. ouachitensis* (Lindeman, 1999). The variables are *G. nigrinoda* – High, Med, and Low and *G. cageli* – Male. All growth curves represent the same duration (0 - 0.75 years).



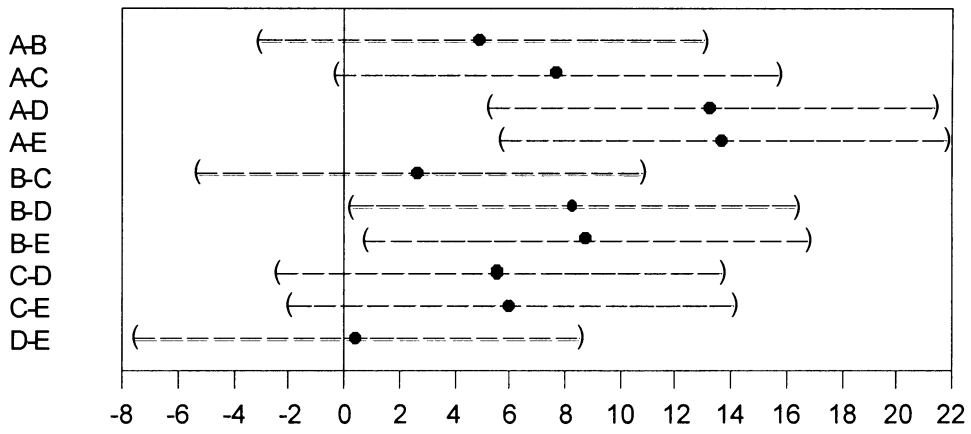
g. Growth curves for the juvenile *Graptemys nigrinoda* data from this evaluation of dietary protein compared to projected growth curves for *G. pseudogeographica* (Lindeman, 1999). The variables are *G. nigrinoda* – High, Med, and Low and *G.pseudogeographica*– Male and Female. All growth curves represent the same duration (0 - 0.75 years).



h. Growth curves for the juvenile *Graptemys nigrinoda* data from this evaluation of dietary protein compared to projected growth curves for *G. versa* (Lindeman, 1999). The variables are *G. nigrinoda* – High, Med, and Low and *G. versa* – Female. All growth curves represent the same duration (0 - 0.75 years).

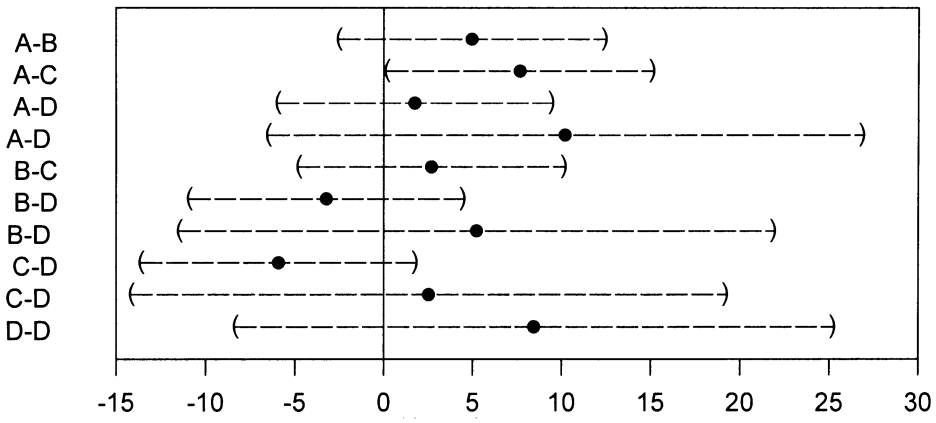
APPENDIX III

Results from Tukey's Multiple Comparison post hoc tests for the evaluation of dietary protein on juvenile *Graptemys nigrinoda*. If the ANOVA for the differences in the mean of the treatments from the morphological or mass character was significant ($P < 0.05$), a Tukey's test was performed. S-Plus software was used to analyze the data and identifies the variables that are different in the ANOVA. The morphological or mass character is identified for each Tukey's Multiple Comparison post hoc test. If the Tukey's test was used for *G. nigrinoda* compared to another *Graptemys sp.* (Lindeman, 1999) both species are provided.



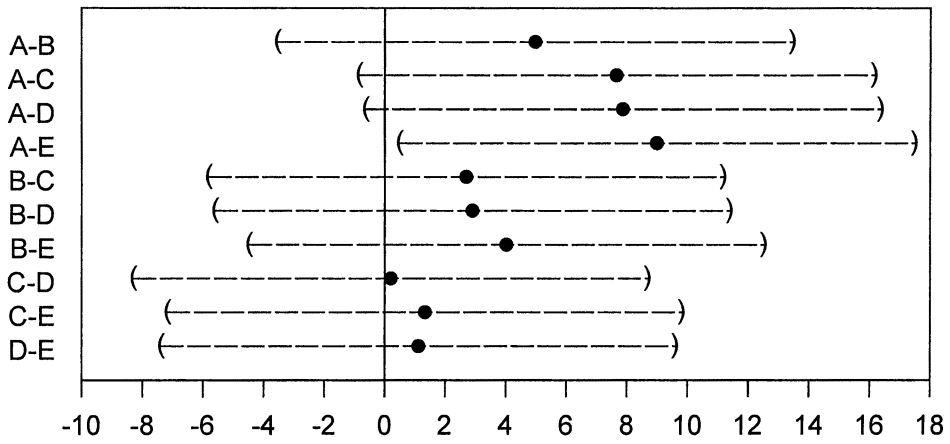
Variables: A = *G. nigrinoda* High, B = *G. nigrinoda* Med, C = *G. nigrinoda* Low, D = *G. ouachitensis* Male, E = *G. ouachitensis* Female.

a. Tukey's Multi ComParison (MCP) post hoc test for *G. nigrinoda* from the evaluation of dietary protein on juvenile turtles and the projected growth curve for *G. ouachitensis* (Lindeman, 1999).



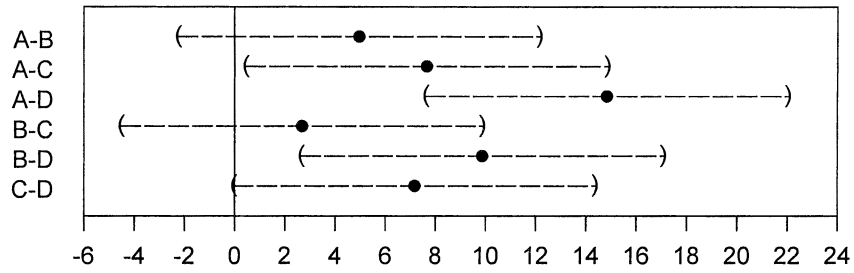
Variables: A = *G. nigrinoda* High, B = *G. nigrinoda* Med, C = *G. nigrinoda* Low, D = *G. cageli* Male

b. Tukey's Multi ComParison (MCP) post hoc test for *G. nigrinoda* from the evaluation of dietary protein on juvenile turtles and the projected growth curve for *G. cageli* (Lindeman, 1999).



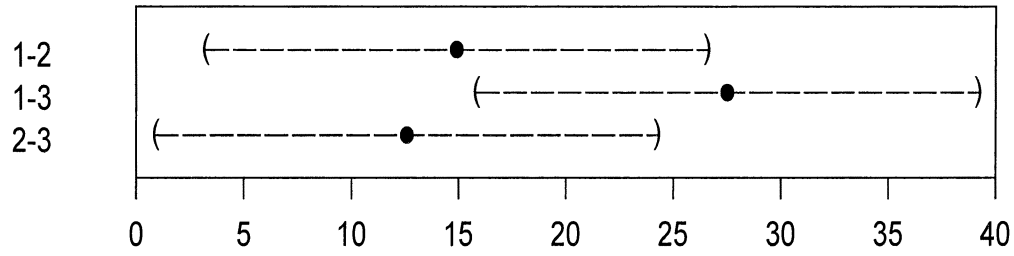
Variables: A = *G nigrinoda* High, B = *G nigrinoda* Med, C = *G nigrinoda* Low, D = *G pseudogeographica* Male, E = *G pseudogeographica* Female.

c. Tukey's Multi ComParison (MCP) post hoc test for *G. nigrinoda* from the evaluation of dietary protein on juvenile turtles and the projected growth curve for *G. pseudogeographica* (Lindeman, 1999).



Variables: A = *G nigrinoda* High, B = *G nigrinoda* Med, C = *G nigrinoda* Low, D = *G versa* Female

d. Tukey's Multi ComParison (MCP) post hoc test for *G. nigrinoda* from the evaluation of dietary protein on juvenile turtles and the projected growth curve for *G. versa* (Lindeman, 1999).

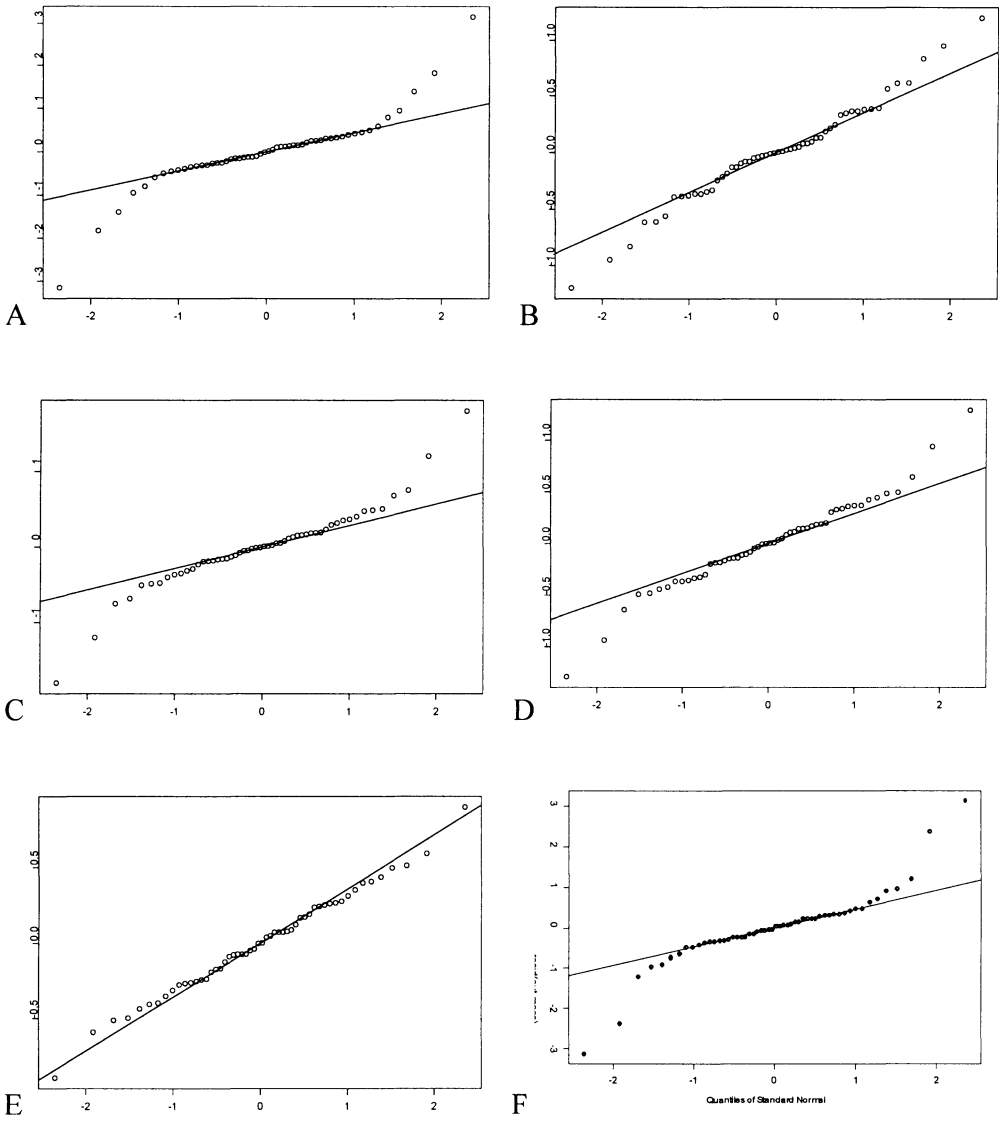


Variables: 1 = *G nigrinoda* High, 2 = *G nigrinoda* Med, 3 = *G nigrinoda* Low

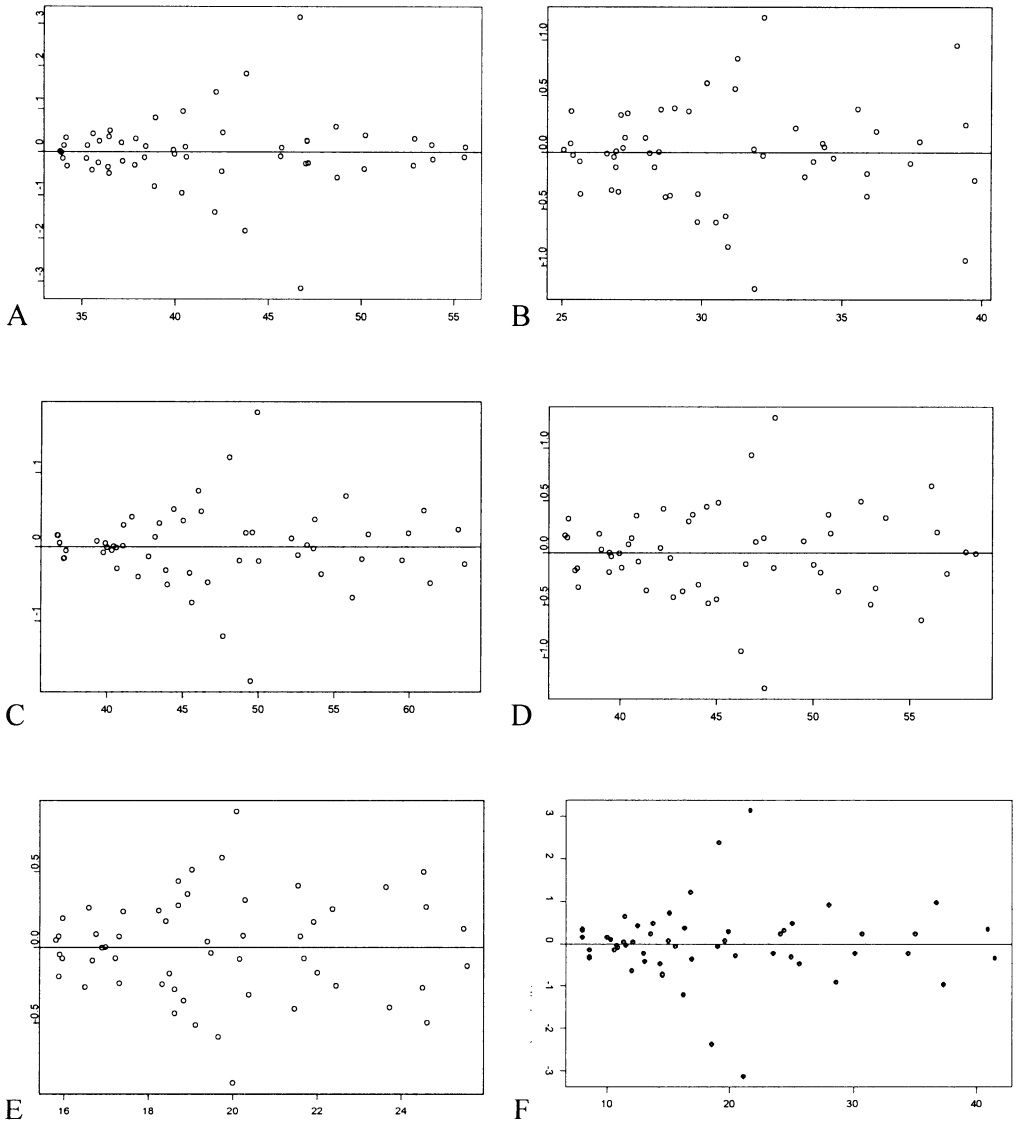
e. Tukey's Multi ComParison (MCP) post hoc test for *G. nigrinoda* from this study and the mortality rate across all diets. This test shows where the differences are in the ANOVA. Because the responses do not overlap 0, there are differences across all groups.

APPENDIX IV

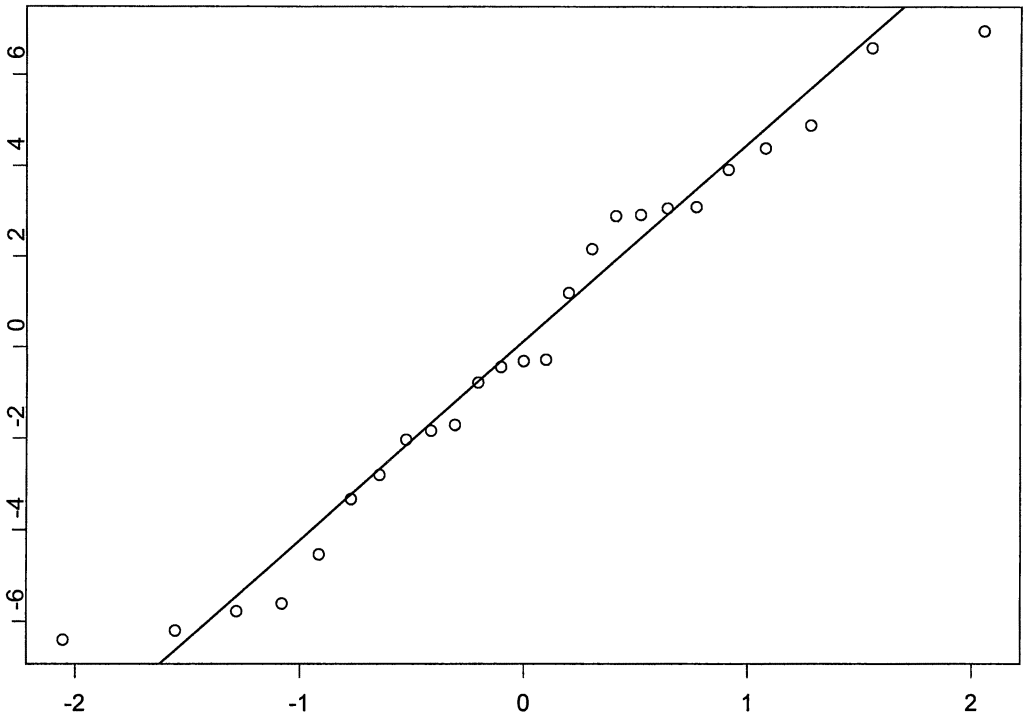
Scatter plots to test the assumptions of normality and homoscedasticity for any and all morphological and mass characters used in the evaluation of dietary protein on juvenile *Graptemys nigrinoda*. Unless otherwise indicated, the data are for the first 9-month phase of the experiment. Assumptions were not violated on any test. Either "Normality" or "Homoscedasticity" will be indicating, depending on the test performed.



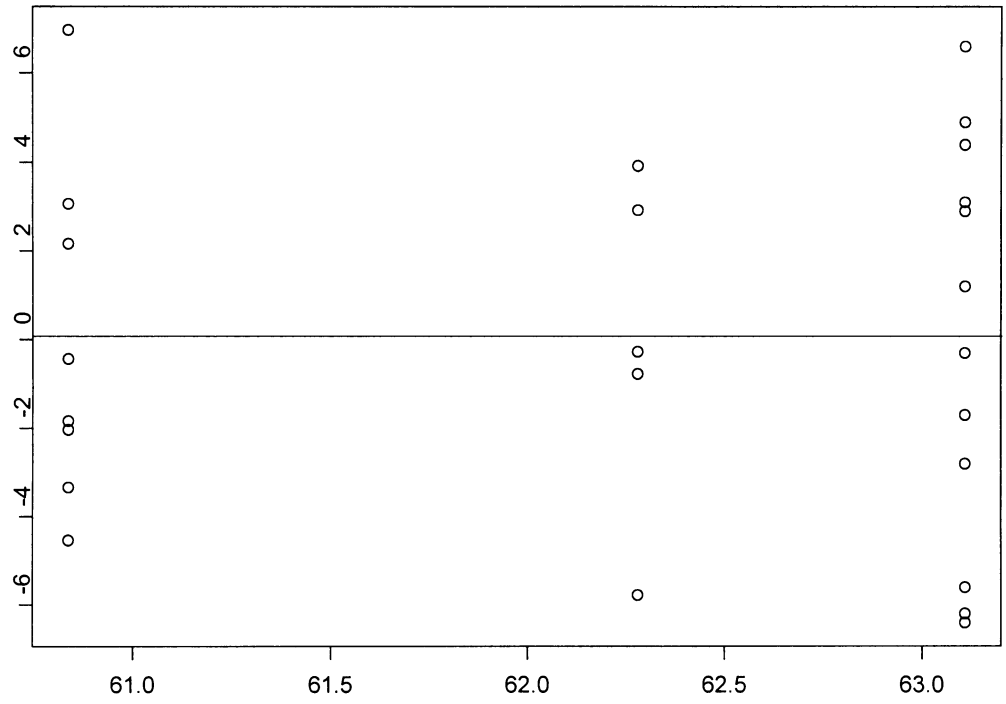
a. Scatter plots for each measurement character to test the assumption of normality. A. plastron length, B. plastron width, C. carapace length, D. carapace width, E. dorso-ventral depth, F. mass. The residuals do not fall far from the line for any measurement; therefore, assumption of normality is met.



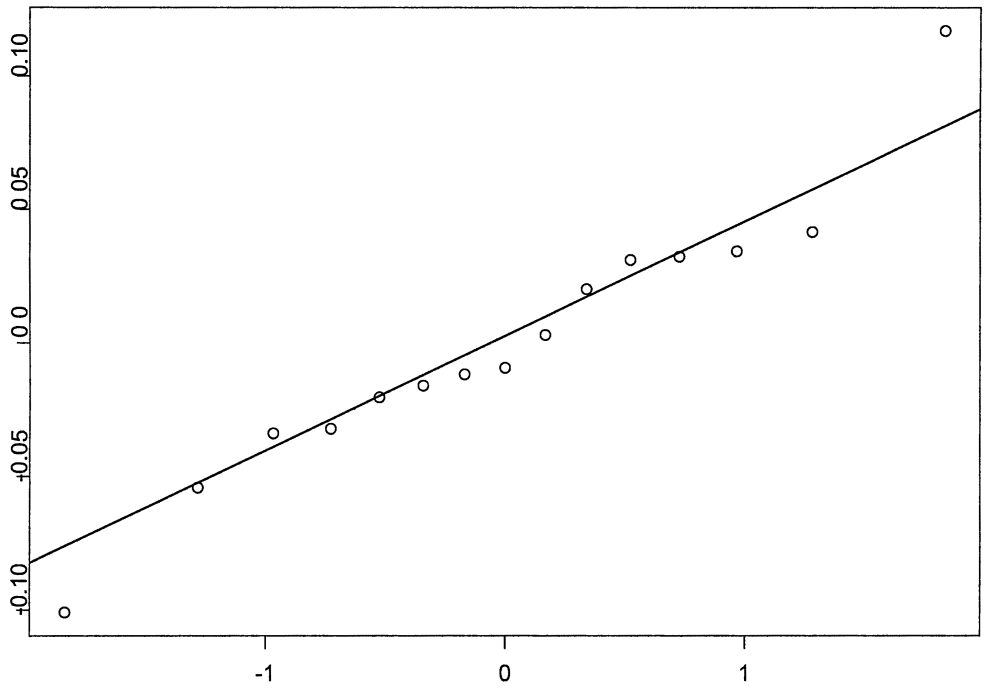
b. Scatter plot to test assumption of homoscedasticity for all measurement characters for the evaluation of variable dietary protein on juvenile *Graptemys nigrinoda*. A. plastron length, B. plastron width, C. carapace length, D. carapace width, E. dorso-ventral depth, F. mass. The residuals do not make any "pattern" and are of equal distribution about the 0 line. Assumption of homoscedasticity is met for all characters.



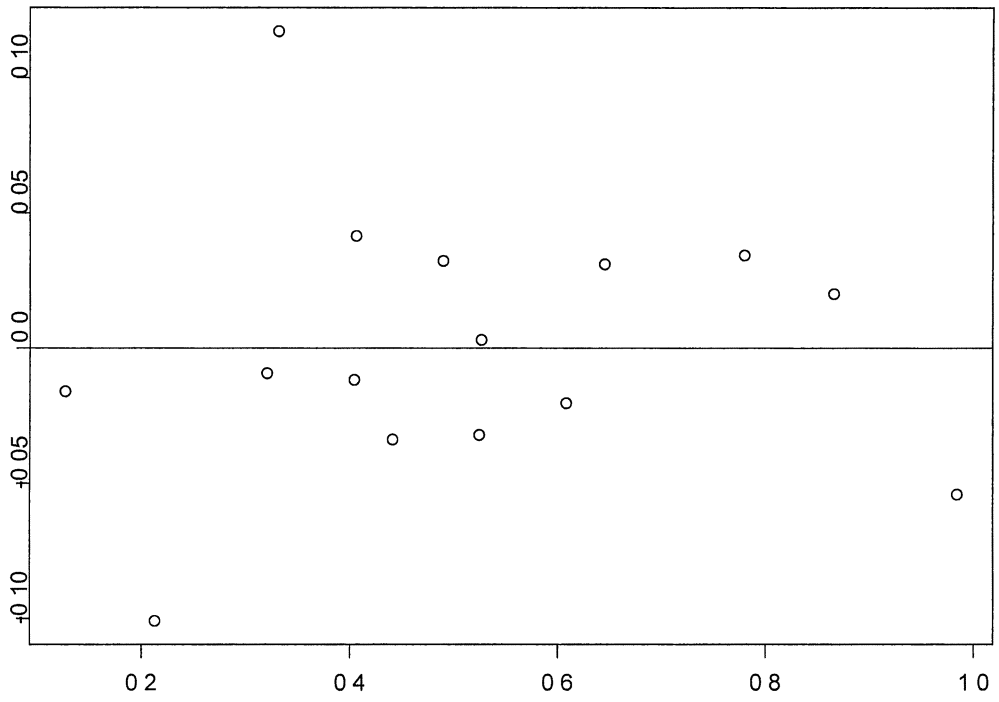
c. Scatter plot to test assumptions for normality for plastron length including both 9-month phases of the evaluation of variable dietary protein on juvenile *Graptemys nigrinoda*. Residuals do not fall far from the line with few "tails." Assumption of normality is met.



d. Scatter plot to test assumptions for homoscedasticity for plastron length including both 9-month phases of the evaluation of variable dietary protein on juvenile *Graptemys nigrinoda*. Residuals do not fall far from the line with few "tails." Assumption of normality is met



e. Scatter plot testing the assumption of normality for mortality across the different diets in the current evaluation of variable protein in juvenile *Graptemys nigrinoda*. The residuals are close to the line with few "tailers". The assumption of normality is not violated.



f. Scatter plot testing the assumption of homoscedasticity for mortality across the different diets in this evaluation of variable protein on juvenile *Graptemys nigrinoda*. The residuals are close to the line with few "tailers". The assumption of homoscedasticiy is not violated

APPENDIX V

Statistical mean ($\overline{X}$) calculated for each diet treatment for each measure period for each morphological or mass measurement in this evaluation of variable dietary protein and its effects on juvenile *Graptemys nigrinoda*.

Meas PD	Diet	PL	PW	CL	CW	Depth	Mass
1	1	33.85	25.46	36.91	37.51	15.91	8.29
2	1	37.15	28.14	41.85	41.10	18.29	12.78
3	1	39.93	29.71	45.25	43.82	19.10	15.31
4	1	43.73	32.02	49.81	47.70	20.34	20.14
5	1	47.01	34.12	53.42	50.60	21.96	24.72
6	1	50.12	36.01	57.01	53.40	23.72	30.35
7	1	52.79	37.57	59.69	55.73	24.51	34.64
8	1	53.85	39.34	61.22	56.66	24.63	37.15
9	1	55.57	39.59	63.49	58.14	25.54	41.21
10	1	62.43	43.02	69.80	63.14	26.28	47.83
1	2	34.01	25.23	37.08	37.40	15.92	8.30
2	2	35.90	27.02	40.21	39.70	17.35	11.06
3	2	36.43	27.20	40.89	40.34	16.72	11.87
4	2	38.39	28.69	43.66	42.49	18.44	14.03
5	2	40.56	30.28	46.40	44.71	19.44	16.60
6	2	42.56	31.08	48.99	46.81	20.21	19.34
7	2	45.70	33.50	52.41	51.03	21.64	23.90
8	2	47.08	34.55	53.92	51.03	21.56	25.32
9	2	48.62	35.71	55.96	52.70	22.38	28.25
10	2	62.48	44.06	70.38	64.40	26.62	49.40
1	3	34.17	25.49	36.96	37.56	15.85	8.33
2	3	35.26	26.76	39.53	39.27	16.94	10.30
3	3	35.57	26.94	40.10	39.16	16.54	10.56
4	3	36.53	27.12	40.85	40.70	17.27	11.71
5	3	37.86	28.27	42.97	42.36	18.67	13.26
6	3	38.99	28.92	44.26	43.57	18.69	14.95
7	3	40.49	30.10	45.91	46.63	18.91	16.69
8	3	42.27	31.19	47.98	46.63	19.74	19.04
9	3	43.86	32.11	49.79	47.82	20.08	21.52
10	3	61.32	42.17	68.28	61.64	25.49	43.22

VITA

Nicole Burpo was born in Houston, Texas, on June 13, 1974 the daughter of Helen Davis and Nicholas Bonrepos. After completing her coursework at Lamar Consolidated High School in Rosenberg, Texas, in 1992, she entered Texas A&M University in College Station, Texas. She received a Bachelor of Science from Texas A&M University in 1996. While pursuing her Master's Degree from Texas State University - San Marcos, she worked as graduate instructional assistant for Genetics and ultimately as Lab Coordinator for Genetics.

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