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The Ecology of Freshwater Turtle Communities on the Upper-Coastal Plain of South Carolina

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THE ECOLOGY OF FRESHWATER TURTLE COMMUNITIES
ON THE UPPER-COASTAL PLAIN OF SOUTH CAROLINA

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Wildlife and Fisheries Biology

by
Patrick Lee Cloninger
August 2007

Accepted by:
Dr. Greg K. Yarrow, Committee Chair
Dr. Peter D. King
Dr. James R. Frederick
Dr. William C. Bridges, Jr

ABSTRACT

A mark-recapture study was used to examine aspects of freshwater turtle ecology, including the size at maturity, sex ratio, growth, sexual size dimorphism and ectoparasite infestation, on the Upper-Coastal Plain of South Carolina at Pee Dee Research and Education Center (PDREC). Turtles were trapped at 8 sites in Dargans' Pond during the summers of 2002, 2004, 2005 and 2006 for a total of 279 trap nights and at 6 sites in the smaller ponds at PDREC for a total of 120 trap nights during the summer of 2006. In Dargans' Pond there were 548 captures of *Trachemys scripta*, made up of 430 turtles and 67 captures of *Sternotherus odoratus* made up of 60 turtles. In the PDREC ponds there were 170 captures of 136 *T. scripta*.

The mean plastron length of *T. scripta* males and females was found to be significantly higher in Dargans' Pond. In Dargans' Pond and PDREC ponds male *T. scripta* were found to mature at a mean plastron length of 102 mm. The adult sex ratio of *T. scripta* (1.20) and *S. odoratus* (1.24) in Dargans' Pond were not significantly different from 1:1. The adult sex ratio of *T. scripta* in PDREC ponds was found to be significantly male-biased (1.70). In Dargans' Pond the growth rate of male *T. scripta* ranged from 0.79-7.84 mm/yr, while female growth rates ranged from 3.19-14.97 mm/yr. In PDREC ponds male growth rates ranged from 0.1-24.9 mm/yr, while female growth rates ranged from 4.98-36.2 mm/yr. The degree of sexual size dimorphism was assessed using a sexual size dimorphism index (SDI). For *T. scripta* inhabiting Dargans' Pond and PDREC ponds the SDI was found to be 1.35 and 1.36. For *S. odoratus* in Dargans' Pond the SDI was 1.05. The only species of leech found parasitizing turtles was *Placobdella*

parasitica. A significant positive relationship was found between the number of leeches parasitizing *T. scripta* and body mass, plastron length, carapace length, and carapace width. Aspects of the life-history and ecology of freshwater turtles are discussed.

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The methods employed in this study were approved by the Clemson University Animal Use Committee. Turtles were collected under Scientific Collecting Permits, numbers 51-2005 and 47-2006, issued by the South Carolina Department of Natural Resources.

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CHAPTER ONE

THE POPULATION STRUCTURE OF *TRACHEMYS SCRIPTA* AND *STERNOTHERUS ODORATUS* ON THE UPPER-COASTAL PLAIN

Abstract

A mark-recapture study was used to examine the population structure of *Trachemys scripta* and *Sternotherus odoratus* on the Upper-Coastal Plain of South Carolina at Pee Dee Research and Education Center (PDREC). Turtles were trapped at 8 sites in Dargans' Pond during the summers of 2002, 2004, 2005 and 2006, totaling 279 trap nights and at 6 sites in the smaller ponds at PDREC (PDREC ponds) totaling 120 trap nights during the summer of 2006. In Dargans' Pond there were 548 captures of *T. scripta*, made up of 430 turtles and 67 captures of *S. odoratus* made up of 60 turtles. In the PDREC ponds there were 170 captures of 136 *T. scripta*. In Dargans' Pond, the observed male to female sex ratio for adult *T. scripta* was 1.20, which was not significantly different from unity. However, the adult sex ratio of *T. scripta* in Dargans' Pond showed a significant annual fluctuation over the 5 years of the study. At 1.24, the adult sex ratio for *S. odoratus* in Dargans' Pond was balanced between males and females. In the PDREC ponds, the observed sex ratio of *T. scripta* was found to be significantly male-biased, at 1.70. The degree of sexual size dimorphism was assessed using a sexual size dimorphism index (SDI). For *T. scripta* inhabiting Dargans' Pond and PDREC ponds, the SDI was found to be 1.35 and 1.36. For *S. odoratus* in Dargans' Pond the SDI was 1.05. The proportion of juvenile to adult *T. scripta* captured in Dargans' Pond and PDREC ponds during 2006 was not significantly different. A significant annual

fluctuation was found in the proportion of juvenile turtles captured over the 5 years of the study. Aspects of the population structure of *T. scripta* and *S. odoratus* are presented.

Introduction

Many freshwater turtle species have been reported to exhibit sexual dimorphism in size. Berry and Shine (1980) described the occurrence of sexual size dimorphism (SSD) in terms of sexual selection, stating that the direction and degree of SSD originated from the mating strategy of males. They theorized that males would be as large as or larger than females in species where male combat and/or forcible insemination is used. In turtle species where female choice is important, selection would favor small males as an adaptation to increase mobility (Berry and Shine, 1980). While, Gibbons and Lovich (1990) argued that SSD must be a response to environmental or physiological differences that are particular to each sex.

The degree and direction of SSD in freshwater turtles has been shown to vary among species and populations. In most species of emydid turtles, females generally reach a larger adult body size than males (Gibbons and Lovich, 1990). Gibbons and Lovich (1990) stated that in all populations of *Trachemys scripta*, females would be larger, a pattern attributed as being a trade-off between delayed maturation and early reproduction. In *Sternotherus odoratus*, the actual direction of SSD has been shown to vary between populations. Tinkle (1961) found males to be as large as or slightly larger than females in northern populations (7.2-12.8° C mean annual temperature) of *S. odoratus*, the sexes in intermediate populations to be equal in size and southern populations (21.1-23.9° C isotherm) to have larger females.

The adult sex ratios observed in freshwater turtle studies can be indicators of ecological processes occurring within a population (Smith and Iverson, 2002). The adult sex ratio is an important demographic measurement because of the potential impact it can have on population dynamics such as, intrasexual competition, time spent searching for mates and egg production (Lovich and Gibbons, 1990). Fisher (1930) originally theorized that parental investment should be distributed equally between male and female offspring, considering a 1:1 sex ratio as being evolutionary stable. Skalski et al. (2005) argued that productivity in polygamous species would not be maximized at a 1:1 sex ratio. Lovich and Gibbons (1990) presented evidence to show that in animal species that differ in the timing of maturity, the sex that matures earliest will predominate numerically.

Research on freshwater turtle species have reported adult sex ratios to vary between species and populations. Cagle (1942) examined 972 *T. scripta* in an Illinois population to find a significantly female biased sex ratio of 0.69. In Mississippi, the sex ratio for 192 *T. scripta* was reported to be significantly male biased at 1.42. Gibbons (1990a) reported the sex ratio for 9 populations of *T. scripta* in South Carolina to range from a female biased 0.31 to a male biased 2.48. Several studies of *S. odoratus* populations have reported the adult sex ratio to be balanced between males and females (Tinkle, 1961; Ernst, 1986; Mitchell, 1988). Cagle (1942) found the sex ratio of a population of *S. odoratus* in Illinois to favor females at 0.32, while a long-term study of an Indiana population found the adult sex ratio to be significantly skewed towards males, at 1.70 (Smith and Iverson, 2002).

The turtles, *T. scripta* and *S. odoratus* are abundant and widespread throughout the Southeastern United States, having been the subjects of numerous studies. However, few studies have attempted to examine their population structure, presumably due to the large sample size required. Therefore, this study was designed to establish sex ratios, examine sexual size dimorphism and the distribution of sizes within two sub-populations of *T. scripta* and a population of *S. odoratus* on the Upper-Coastal Plain of South Carolina, an area with no record in the literature of previous chelonian studies.

Methods

The study was conducted at Pee Dee Research and Education Center near Florence, South Carolina. Turtles were trapped using 1.2 m x .9 m hoop-nets with 2.5 cm nylon mesh, baited with canned cat food. Cans were punctured and hung from the center of the trap so that the bait hung between the entrance and the bottom of the trap. Traps were set parallel to the shore for a 24 hour period. Trapping was conducted in Dargans' Pond during the summers of 2002, 2004, 2005 and 2006 and in the six smaller ponds during the summer of 2006. In Dargans' Pond turtles were trapped at 8 sites for a total of 279 trap nights and at 6 sites in the small ponds totaling 120 trap nights.

Once captured, turtles were identified to species, sexed and weighed using an electronic balance (to the nearest g). Maximum straight-line plastron and carapace length and carapace width were measured using calipers (to the nearest mm). Individual turtles were marked using AVID® PIT (Passive Integrated Transponder) tags. Once measurements were taken, turtles were scanned with an AVID® PIT tag reader in order to determine if they have been previously captured. New captures were then marked by

using a syringe to insert a PIT tag into the left inner thigh between the skin and muscle tissue. Individuals weighing less than 100g were marked, following Cagle (1939), by notching the marginal scutes. As individual turtles were processed they were immediately released at the trap site from which they were captured.

The display of secondary sexual characteristics was used to identify maturity in turtles. In Dargans' Pond and PDREC ponds, the 10 smallest (plastron length) male *Trachemys scripta* displaying secondary sexual characteristics were averaged in order to determine the mean size at maturity. Female *T. scripta* were considered sexually mature at a plastron length of 160 mm (Gibbons and Greene, 1990). Maturity in *Sternotherus odoratus* males was identified through the presence of scale patches on the rear legs, an enlarged tail base, and separation of plastral scutes (Risley, 1930). In *S. odoratus*, males were considered mature at a plastron length of 54 mm and females were considered mature at 58 mm (Mitchell, 1988).

The degree of sexual size dimorphism was assessed using the sexual size dimorphism index ($SDI = \text{mean plastron length of adult females} / \text{mean plastron length of adult males}$) developed by Gibbons and Lovich (1990). The SDI is assigned to be positive when females are the larger sex and negative when males are the larger sex. An SDI was calculated for *S. odoratus* and *T. scripta* in Dargans' Pond and for *T. scripta* in PDREC ponds.

The functional adult sex ratio was estimated for *S. odoratus* and *T. scripta* in Dargans' Pond and for *T. scripta* in PDREC ponds. Statistical data analyses were performed using SAS, version 9.1.3 (2000-2004). Significance was assumed at P-value <

0.05. Chi-square tests were used to determine if the observed male to female sex ratios differed from 1:1. A chi-square contingency table was used to determine if there was an annual fluctuation in the sex ratio of *T. scripta* in Dargans' Pond over the 5 year study period.

For *T. scripta*, the proportion of adults to juveniles captured was used to examine reproduction (Skalski et al., 2005). The observed proportion of juveniles to adults for captures during 2006 was calculated for *T. scripta* in Dargans' Pond and PDREC ponds. These proportions were compared using a chi-square test. A chi-square contingency table was used to determine if there was an annual fluctuation in the proportion of juveniles to adults for *T. scripta* in Dargans' Pond.

The seasonal activity of *T. scripta* in Dargans' Pond was compared between males and females using chi-square tests for each month. A chi-square test was also used to determine if the hoop nets used to capture turtles were male-biased by combining the total number of male and female *T. scripta* captures in Dargans' Pond.

Results

The 4 years of the study yielded 802 turtle captures made up of 565 individuals. In Dargans' Pond there were 622 captures of 413 turtles made up of 4 species, being *Kinosternon subrubrum*, *Psuedemys concinna*, *Sternotherus odoratus* and *Trachemys scripta* (Table 1.1). In the small ponds at PDREC there were 180 captures of 152 turtles made up of 6 species, being *Chelydra serpentina*, *Kinosternon baurii*, *K. subrubrum*, *P. concinna*, *S. odoratus* and *T. scripta* (Table 1.2).

Table 1.1. Turtle species captured in Dargans' Pond, including the number of captures, the number of individuals captured and the number of individuals recaptured.

Species	Total Captures	Individuals Captured	Individuals Recaptured
<i>Kinosternon subrubrum</i>	6	5	1
<i>Psuedemys concinna</i>	1	1	0
<i>Sternotherus odoratus</i>	67	60	6
<i>Trachemys scripta</i>	548	413	73

Table 1.2. Turtle species captured in the small ponds at PDREC, including the number of captures, the number of individuals captured and the number of individuals recaptured.

Species	Total Captures	Individuals Captured	Individuals Recaptured
<i>Chelydra serpentina</i>	2	2	0
<i>Kinosternon bairii</i>	1	1	0
<i>Kinosternon subrubrum</i>	1	1	0
<i>Psuedemys concinna</i>	5	4	1
<i>Sternotherus odoratus</i>	11	11	0
<i>Trachemys scripta</i>	170	136	29

In Dargans' Pond, the mean plastron length at which *T. scripta* males were found to mature was 102.6 mm (range: 89-112) and in PDREC ponds, males matured at a mean plastron length of 102.1 mm (range: 92-116), which were not significantly different ($t = 0.13$, $df = 18$, $P = 0.90$).

In Dargans' Pond a total of 354 adult *T. scripta* were captured over the course of the study. Of these, 194 were males and 162 were females, yielding a male to female sex ratio of 1.20, which was not significantly different ($\chi^2 = 2.88$) from the hypothetical 1:1

sex ratio. The observed sex ratio of *T. scripta* captured in Dargans' Pond showed significant annual fluctuation ($\chi^2 = 13.15$, $df = 3$, $P < 0.01$) (Table 1.3). The 56 adult *S. odoratus*, captured in Dargans' Pond, were composed of 31 males and 25 females. The estimated male to female sex ratio of 1.24 for *S. odoratus* was not significantly different than 1:1 ($\chi^2 = 0.64$). In PDREC ponds a total of 105 adult *T. scripta* were captured over the course of the study. Of these, 66 were males and 39 were females, yielding a male to female sex ratio of 1.70, which was significantly different ($\chi^2 = 6.94$) from the theoretical 1:1 sex ratio.

Table 1.3. The observed sex ratio of *Trachemys scripta* in Dargans' Pond based on the number of individual male and female turtles captured during each year of the study.

Year	Male	Female	Sex Ratio
2002	37	22	1.68
2004	43	43	1.00
2005	17	24	0.71
2006	96	72	1.33

Sexually mature *T. scripta* males captured in Dargans' Pond ranged from 105-227 mm in plastron length with a mean of 154.4 mm and mature females ranged from 160-265 mm with a mean of 208.3 mm (Figure 1.1). Therefore, the SDI for *T. scripta* captured in Dargans' Pond was 1.35.

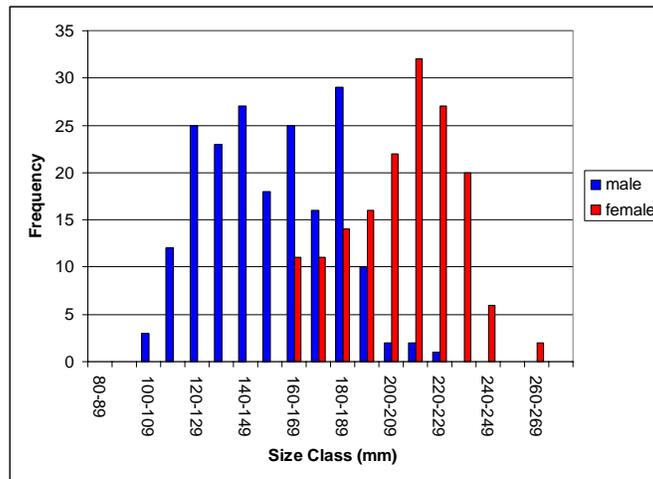


Figure 1.1. Frequency distributions of 10-mm size classes based on the plastron length of 354 adult *Trachemys scripta* captured in Dargans' Pond.

Sexually mature *S. odoratus* males captured in Dargans' Pond ranged from 55-78.5 mm in plastron length with a mean of 64.3 mm and females ranged from 56-92 mm with a mean plastron length of 67.4 (Figure 1.2). This produced an SDI of 1.05.

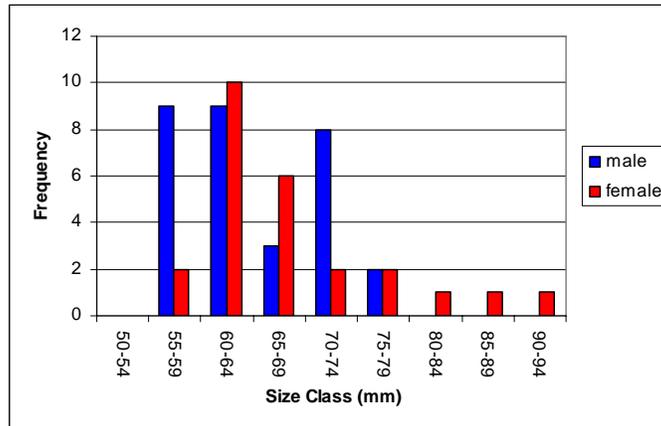


Figure 1.2. Frequency distributions of 5-mm size classes based on the plastron length of 56 adult *Sternotherus odoratus* captured in Dargans' Pond.

Sexually mature *T. scripta* males captured in PDREC ponds ranged from 104-205 mm in plastron length with a mean of 144.8 and females ranged from 163-230 mm with a mean plastron length of 196.5 mm (Figure 1.3). Therefore, the SDI for *T. scripta* captured in PDREC ponds was 1.36.

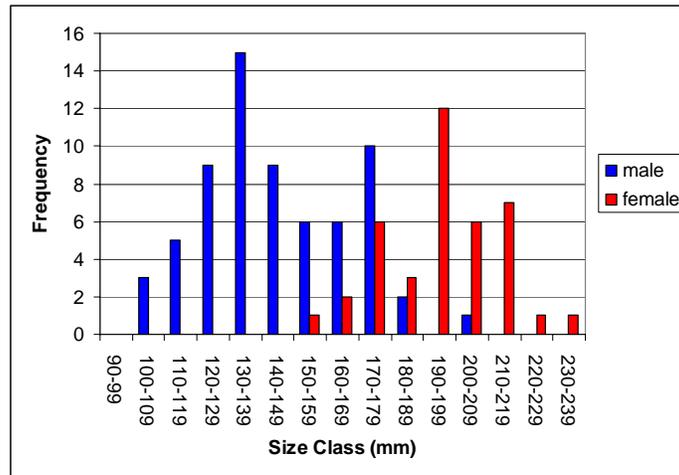


Figure 1.3. Frequency distributions of 10-mm size class based on the plastron length of 105 adult *Trachemys scripta* captured in the PDREC ponds.

All *T. scripta* captured in Dargans' Pond during the first year of the study (2002) were identified as adults. In 2004, 2005 and 2006, juveniles accounted for 26%, 10%, and 20% of *T. scripta* captured (Table 1.4). When considering all four years, there was a significant ($\chi^2 = 14.92$, $df = 3$, $P < 0.01$) annual fluctuation in the proportion of *T. scripta* juveniles to adults. However, there appears to be no significant difference ($\chi^2 = 3.04$, $df = 3$, $P = 0.22$) in the proportion of juveniles to adults between 2004, 2005 and 2006. In PDREC ponds, the observed proportion of *T. scripta* juveniles to adults during 2006 was 30%, which was not significantly different ($\chi^2 = 2.14$, $df = 1$, $P = 0.14$) than that of Dargans' Pond for 2006 (20%).

Table 1.4. The observed proportion (π_j) of juveniles to adults for *Trachemys scripta* captured in Dargans' Pond during each year of the study.

Year	Juveniles	Adults	π_j
2002	0	59	0.00
2004	22	86	0.26
2005	4	41	0.10
2006	33	168	0.20

In Dargans' Pond, there was no difference in the number of male and female *T. scripta* captures during May ($\chi^2 = 2.47$), June ($\chi^2 = 0.31$), August ($\chi^2 = 3.51$), September ($\chi^2 = 3.52$), and October ($\chi^2 = 1.32$). A significant difference was found in the number of male and female captures in July ($\chi^2 = 3.95$), with males being the numerically dominant sex. There was no significant difference between the total number of male and female *T. scripta* captured in hoop nets during the four years of sampling ($\chi^2 = 2.49$).

Discussion

In natural history studies, it is necessary to determine the size at which each sex reaches maturity in a population. In both PDREC ponds and Dargans' Pond, male *Trachemys scripta* were found to mature at a mean plastron length of 102 mm. Similarly, Gibbons and Greene (1990) noted, that in two populations of *T. scripta* at the Savannah River Plant, near Aiken, South Carolina, males began to attain maturity at a plastron length of 100 mm. Because female turtles were not palpated for eggs during the study it was not possible to determine the size at which they mature sexually. The size at maturity must be established in order to estimate functional adult sex ratios and sexual

dimorphism indices (Gibbons, 1990a; Gibbons and Lovich, 1990). Gibbons and Greene (1990) found female *T. scripta*, in several South Carolina populations, to mature at a plastron length of 160 mm. Based on their results, female *T. scripta* in our study were assumed to be mature at a plastron length of 160 mm. In *Sternotherus odoratus*, males were considered mature at a plastron length of 54 mm and females were considered mature at 58 mm (Mitchell, 1988).

The sex ratios established in this study only represent the observed sex ratio for the turtles captured. It is not known whether the observed sex ratios are representative of these turtle populations. Gibbons (1970a) noted that an important assumption in studies concerning sex ratios is that the sampling is not biased. In a study of *Chrysemys picta*, Ream and Ream (1966) found that different sampling methods resulted in different size-class distributions and sex ratios. Similarly, Gibbons (1990a) noticed that aquatic traps were selective towards males, whereas terrestrial traps were selective towards females. Yet, other studies have found no difference in the observed sex ratios of turtle populations using a variety of different capture methods (Lovich and Gibbons, 1990; Smith and Iverson, 2002; Barko and Briggler, 2006). In this study, there was no significant difference in the total number of male and female *T. scripta* captured in hoop nets during the four years of sampling in Dargans' Pond. Based on these results, the idea that hoop nets produce male-biased captures is dismissed for the purposes of this study.

In natural populations of *T. scripta*, the observed adult sex ratio has been found to vary from strongly female biased (59% female; Cagle, 1942) to strongly male biased (78% male; Webb, 1961). However, in *T. scripta* populations, observed sex ratios

deviating from 1:1 are usually skewed towards males (Gibbons, 1990a). Lovich and Gibbons (1990) predicted that in species that differ in age at maturity, the early maturing sex would predominate numerically. Therefore, all else equal, in populations of *T. scripta* there should be a consistent surplus of males. However, a surplus of males may compete with females for resources; thus, maximum productivity would be favored by fewer males and a surplus of females (Skalski et al., 2005).

In Dargans' Pond the observed adult sex ratio for adult *T. scripta* was 1.20 (54% male), therefore, as expected, males were the numerically dominant sex, yet, not to the point where the sex ratio was significantly different from unity. However, in PDREC ponds, the observed sex ratio of *T. scripta* was significantly male biased, at 1.70 (63% male). In polygamous species, a complex demographic relationship exists between sex ratio and productivity, and therefore sex ratios deviating from 1:1 deserve an explanation (Smith and Iverson, 2002; Skalski et al., 2005). Many theories have been offered in an attempt to explain why sex ratios in natural populations deviate from 1:1. Gibbons (1990a) identified four demographic characteristics that influence sex ratios in turtles as being: the sex ratio of hatchlings, differential mortality of the sexes, differential emigration and immigration and differential age at maturity.

Even though turtles in natural populations have a high potential for longevity, mortality rates are high enough in most species to cause a complete population turnover within 30 years (Gibbons, 1987). In general, freshwater turtles experience considerable mortality, primarily through predation, in eggs, hatchlings and small juveniles, with relatively low and constant mortality at the large juvenile and adult stages (Gibbons and

Semlitsch, 1982; Iverson, 1991). In some species, differential activity or behavioral patterns can lead to seasonal variation in mortality rates among sexes (Gibbons, 1990a; Thomas et al., 1999). In *T. scripta*, male mating activity has been reported to peak in early spring and late fall, when males increase mate searching activities, including long overland tracks, in order to locate receptive females (Morreale et al., 1984; Gibbons, 1990b). Female activity peaks in early summer, when they leave aquatic habitats to nest (Morreale et al., 1984; Gibbons, 1990b). In our study, turtles were trapped in Dargans' Pond from May through October and July was the only month in which there was a significant difference between the number of male and female captures of *T. scripta*. Therefore, turtle captures did not necessarily reflect the peak activity seasons identified for male and female *T. scripta*. Based on this evidence, differential activity patterns between the sexes is not assumed to effect the observed sex ratio of *T. scripta* in this study.

Differential mortality of the sexes, where one sex survives better than the other, has been implicated as a possible cause of biased sex ratios in turtle populations (Smith and Iverson, 2002). However, this has only been reported for a few populations. In *S. odoratus*, the annual survival rate has been found to be highest among males (Edmonds and Brooks, 1996), whereas survivorship in *T. scripta* has been found to be highest in females (Parker, 1996). However, the majority of studies have reported annual survivorship to be similar between the sexes (*S. odoratus* and *C. picta* Mitchell, 1988; *T. scripta*, Frazer et al., 1990; *Emydoidea blandingii*, Germano et al., 2000). In one study, a population of *Clemmys guttata* was found to have a balanced sex ratio (1.08) despite

survivorship being slightly higher among females (Litzgus, 2006). If differential mortality was responsible for the male-biased sex ratio in PDREC ponds then the sex ratio of *T. scripta* in Dargans' Pond should reflect this due to their close proximity. Therefore, it is highly unlikely that differential mortality is responsible for the male-biased sex ratio observed for *T. scripta* inhabiting PDREC ponds.

Many reptile species, particularly chelonians, exhibit temperature-dependent sex determination (Vogt and Bull, 1984; Bull, 1985; Wibbels et al., 1998). In *T. scripta*, sex is determined by the incubation temperature during the middle trimester of embryonic development (Wibbels et al., 1991). At incubation temperatures above 29.5°C hatchlings are all female, at temperatures below 29°C hatchlings are all males and temperatures between 29-29.5°C produce both sexes (Bull et al., 1982). In a study of three species of *Graptemys*, with a similar pattern of sex determination, the sex ratio of hatchling turtles was found to be skewed towards females (0.25) in a population that an earlier study observed adult sex ratios to be female-biased (Vogt, 1980). Based upon this evidence, the male-biased sex ratio observed from *T. scripta* in PDREC ponds could be due to a skewed primary sex ratio.

Difference in the timing of maturity between the sexes has been implicated as a cause of skewed sex ratios (Lovich and Gibbons, 1990). In *T. scripta*, males mature at an age of two to five years and females mature at an age of six to ten years (Cagle, 1950; Gibbons et al., 1981). If there was no differential mortality, immigration or emigration and hatchling sex ratios were balanced between males and females, then each year a surplus of male turtles would be recruited into the breeding population. Therefore, it is

possible that the male-biased sex ratio of *T. scripta* inhabiting PDREC ponds is due to the difference in the age at which maturity is attained between the sexes.

In turtles, the fluctuation of sex ratios over time has been attributed to differential immigration and emigration of the sexes (Lovich and Gibbons, 1990). This may explain why there was significant, annual, fluctuation in the observed sex ratio of *T. scripta* in Dargans' Pond. However, *T. scripta* is a species known to exhibit remarkable year-to-year site fidelity (Morreale et al., 1984). In this study, many turtles, both males and females, were recaptured at the same trap site from which they were originally captured for as many as three consecutive years. Together, between Dargans' Pond and PDREC ponds, 549 individual *T. scripta* were collected and marked, yet none were found to move between these two study sites. Based on these results, it is believed that neither differential immigration nor emigration, of the sexes, can explain the annual fluctuation in the observed sex ratio of *T. scripta* in Dargans' Pond or the male-biased sex ratio for *T. scripta* in PDREC ponds.

In turtles, fluctuating sex ratios over time have also been attributed to an unbalanced sex ratio in hatchlings (Lovich and Gibbons, 1990). Lovich and Gibbons (1990) proposed that "variation in nest site selection and environmental temperatures during a female's reproductive life will tend to balance out any differences in year-to-year hatchling sex ratios." In *C. picta*, a single female's offspring was found to vary from all male to all female, yet, the combined hatchling sex ratio of her offspring, over a five year period, was not different from unity (Zweifel, 1989). In this study, a significant annual fluctuation was observed in the adult sex ratio of *T. scripta* inhabiting Dargans'

Pond, yet, the combined sex ratio for the five years of the study was balanced between males and females. Therefore, it is possible that the observed annual fluctuation in the sex ratio was due to unbalanced hatchling sex ratios. However, a more logical explanation would be that the reduced sampling effort in 2002 and 2005 may have produced the annual fluctuation observed in the sex ratio of *T. scripta* in Dargans' Pond.

In turtles, sexual-size dimorphism (SSD) has often been interpreted as a product of differential selection pressures between the sexes. For example, it has been well documented that *T. scripta* males reach maturity at a much smaller size than females, suggesting a dual adaptive strategy between the sexes (Tinkle, 1961; Gibbons et al., 1981). In female *T. scripta* a positive correlation exists between body size and clutch size (Gibbons, 1970b) and therefore a delay in sexual maturity, resulting in a larger body, would increase fecundity (Brooks et al., 1992). In male *T. scripta*, early-maturation, resulting in a smaller body size, permits individuals to enter the breeding population earlier in life allowing energy to be reallocated for mate searching and courtship behavior (Congdon et al., 1982). Therefore, opposing selection pressures in some turtle populations create the foundation for SSD.

As with many of the emydid turtles, the adult body size of *T. scripta* females is significantly larger than that of males in all populations (Gibbons and Lovich, 1990). In Dargans' Pond and PDREC ponds the sexual size dimorphism index (SDI) was found to be 1.35 and 1.36, which fall within the 1.15 and 1.55 range identified by Gibbons and Lovich (1990), for large sample sizes of *T. scripta* populations.

In two populations of *T. scripta* in South Carolina, the mean plastron length of females was approximately 48 mm larger than those of males (Gibbons and Lovich, 1990). In this study, the mean plastron length of *T. scripta* females was 54 mm larger than those of males in Dargans' Pond and 52 mm larger than those of males in PDREC ponds. This relationship between male and female body sizes in *T. scripta* suggests that males may need to reach a minimum size in order to successfully mate with, the larger, females.

Interestingly, the reported SDI for *S. odoratus* populations have ranged from 1.05, where females are the larger sex to -1.07, where males are the larger sex (Tinkle, 1961; Mahmoud, 1967; Gibbons and Lovich, 1990). In the population of *S. odoratus* inhabiting Dargans' Pond, the SDI was found to be 1.05, in which females were the largest sex. Tinkle (1961) and Berry and Shine (1980) found the body size of *S. odoratus* to be slightly larger in northern turtles when compared to those of southern populations. However, Gibbons and Lovich (1990) reported the body size of two populations of *S. odoratus* in South Carolina to be relatively large, with females having a mean plastron length of 86 and 101 mm and males having a mean plastron length of 83 and 98 mm. In Dargans' Pond, the mean plastron length for males and females was found to be 64.3 mm and 67.4 mm. In Virginia, the mean plastron length for male and female *S. odoratus* was found to be 53.7 mm and 58.4 mm (Mitchell, 1988). Based on this evidence, the mean body size of *S. odoratus* is most likely due to factors such as habitat suitability rather than geographic location.

The distribution of sizes within a population can offer valuable inferences into the history and general health of a population. A population of *T. scripta* on Capers Island, South Carolina was found to have no juvenile recruitment from 1978 to 1986, due to alligator (*Alligator mississippiensis*) predation (Gibbons et al., 1979). This produced a population containing only adult turtles, which threatens the persistence of the species on the island (Gibbons et al. 1979). In this study, the proportion of individual *T. scripta* juveniles, during 2006, made up 20% and 30% of the individuals captured in Dargans' Pond and PDREC ponds. In two populations of *T. scripta* studied in Illinois, juveniles made up 18% and 37% of the individuals captured. Therefore, it could be assumed that the *T. scripta* population in both Dargans' Pond and PDREC ponds are successfully reproducing. The 10-mm plastron size distribution charts for *T. scripta* in Dargans' Pond and PDREC ponds show no major gaps between size classes for either sex, suggesting that juvenile recruitment has remained relatively stable in this population. During the five year duration of this study, only four juvenile *S. odoratus* were captured in Dargans' Pond. This is most likely due to their relatively small size, giving them the ability to escape from traps.

In Dargans' Pond, the proportion of individual *T. scripta* juveniles captured showed a significant annual fluctuation. This is primarily due to the fact that no juvenile turtles were captured during the first year (2002) of the study. When compared for the following three field seasons, no significant fluctuation occurred in the proportion of juveniles captured. Therefore, the lack of juvenile presence, in 2002, deserves special consideration.

Extreme environmental conditions have been found to influence reproduction in turtles. Gibbons et al. (1983) found that the number of female *T. scripta* carrying clutches had been reduced by, up to, 86% during a severe drought year. Similarly, in our study area, there was mild to extreme drought conditions from August 1998 to July 2002 (NOAA), which may have severely reduced offspring production and may explain why there were no juveniles captured during the 2002 field season. Because turtles in the hatchling stage are seldom caught in hoop nets, a decrease in offspring production would not be visible for approximately three years (Cagle, 1950; Gibbons et al., 1981). Therefore, drought conditions present at the study site from August 1998 to July 2002 may account for the low proportion of juvenile turtles observed during 2002 (0%) and 2005 (10%). However, if this were the case, the observed proportion of juveniles during the 2004 field season should have been equally low, yet, it was the highest observed during the study (26%). Based on these results, it is highly unlikely that the drought conditions present at the study site produced the low proportion of juvenile captures observed during the 2002 and 2005 field seasons.

Due to their cryptic behavior, juvenile and hatchling turtles are commonly underrepresented in turtle studies and therefore, the proportion of juvenile turtles captured may be directly associated with sampling effort (Ream and Ream, 1966; Ernst, 1976; Litzgus, 2006). In Dargans' Pond, there were a total of 17 trap nights during 2002, which produced no juvenile turtles. While, the highest proportion of juveniles (26%) was captured during the 2004 field season, with 72 trap nights. In 2006, with over double the number of trap nights (160) in 2004, the observed proportion of juveniles was 20%.

Therefore, it could be possible that the reduced sampling effort in 2002 and 2005 was responsible for the low number of juvenile captures observed during those years.

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CHAPTER TWO

GROWTH, BODY SIZE, AND MATURITY IN TWO SUB-POPULATIONS OF *TRACHEMYS SCRIPTA*

Abstract

A mark-recapture study was used to examine growth, body size and maturity in freshwater turtles on at Pee Dee Research and Education Center (PDREC) the Upper-Coastal Plain of South Carolina. Turtles were trapped at 8 sites in Dargans' Pond during the summers of 2002, 2004, 2005 and 2006 for a total of 279 trap nights and at 6 sites in the smaller ponds at PDREC (PDREC ponds) for 120 trap nights during the summer of 2006. In Dargans' Pond there were 548 captures of *Trachemys scripta* made up of 413 turtles. In the smaller ponds there were 170 captures of 136 *T. scripta*. The mean plastron length of both males and females were significantly higher in Dargans' Pond. In both areas, *T. scripta* males were found to mature around 102 mm plastron length. In Dargans' Pond, the growth rate of males ranged from 0.79-7.84 mm/yr, while female growth rates ranged from 3.19-14.97 mm/yr. In PDREC ponds, the growth rate of males ranged from 0.1- 24.9 mm/yr, while female growth rates ranged from 4.98-36.2 mm/yr. A correlation analysis revealed a significant, negative relationship between the growth rates of *T. scripta* and body size for males, females, males and females in Dargans' Pond and males and females in the smaller PDREC ponds. An analysis of covariance (ANCOVA) model was used to compare the growth rates between the sexes, in Dargans' Pond and PDREC ponds, while adjusting for plastron length. In Dargans' Pond, male and female *T. scripta* showed no difference in growth rates at 100 and 200 mm plastron lengths, although,

female growth rates were found to be significantly higher than those of males at plastron lengths 120, 140, 160 and 180 mm. In PDREC ponds, male and female *T. scripta* showed no difference in growth rates at 120 mm plastron length, although, female growth rates were significantly higher than those of males at 100, 140, 160, 180, and 200 mm plastron lengths. An ANCOVA model was also used to compare the growth rates of males and females between Dargans' Pond and PDREC ponds. The growth rates of male *T. scripta* were significantly higher in PDREC ponds than those in Dargans' Pond at plastron lengths of 100, 120, and 140 mm. At a plastron length of 160 mm, the growth rates of male *T. scripta* did not differ between the two areas, while male growth rates were higher in Dargans' Pond at a plastron length of 180 mm. The growth rates of female *T. scripta* in PDREC ponds were found to be significantly higher than those in Dargans' Pond at plastron lengths of 100, 120, 140, 160 and 180 mm, while female growth rates did not differ between the two areas at a plastron length of 200 mm. The factors found to influence growth, maturity and body size in *T. scripta* are presented.

Introduction

Turtles are ideal organisms for studies of vertebrate growth because their rigid shells do not change in size over short-term fluctuations in health. Like all reptilians, chelonian growth is believed to be indefinite, occurring throughout the life-history of the individual (Wilbur, 1975; Shine and Iverson, 1995). Most freshwater turtle species have been shown to follow a similar pattern of growth. The juvenile life-stage is characterized by rapid growth and as turtles increase in size the growth rate steadily decreases (Cagle, 1946; Ernst, 1975; Wilbur, 1975; Bury, 1979; Wilbur and Morin, 1988). The initial

leveling of growth corresponds to the attainment of sexual maturity (Cagle, 1946; Ernst, 1975). In female *Chelydra serpentina*, a significant decrease in growth rate was found to occur during the initial enlargement of ovarian follicles and again at first oviposition (Galbraith et al., 1989). In species exhibiting sexual size dimorphism, such as *Trachemys scripta*, where females reach a much larger size than males, the absolute growth rates of females must be consistently higher than those of males (Dunham, 1978), while the growth rates of juveniles between the two sexes do not vary (Dunham and Gibbons, 1990; Wallin and Gibbons, 1990). In larger, older individuals, growth continues at a low rate and in an inconsistent manor (Dunham and Gibbons, 1990).

The growth of freshwater chelonians has been shown to be dependent on several factors including variation in hatchling size (Mahmoud, 1969), genetics (Mahmoud, 1969), food availability and quality of diet (Mahmoud, 1969; Parmenter, 1980; MacCulloch and Secoy, 1985), habitat suitability (Mahmoud, 1969) and environmental temperature (Cagle, 1946; Pamenter, 1980). Congdon (1989) recognized that in northern latitudes, growth rates may be constrained by the rate at which food can be processed and passed through the gut. Therefore, turtles inhabiting warmer regions would be able to process and harvest more food, leading to an elevated growth rate (Parmenter, 1980; Frazer et al., 1991). These factors, whether acting independently or in combination, create a wide range of growth rates between individuals of the same sex and size (Cagle, 1946; Bury, 1979; Andrews, 1982; Dunham and Gibbons, 1990).

Natural selection has been implicated to play a major role in the growth rate of freshwater chelonians. Werner and Gilliam (1984) identified growth rate as “the primary

characteristic affecting body size and potentially subject to evolutionary change through natural selection.” Many freshwater turtle species experience sexual dimorphism in size and age at maturity, suggesting a dual adaptive strategy between the sexes (Tinkle, 1961; Gibbons et al., 1981). In *T. scripta*, it has been documented that males reach maturity at a certain size whereas females reach maturity at a fixed age (Gibbons et al. 1981). This suggests that an accelerated male growth rate would decrease the time to maturity, while increasing fitness at an early age (Gibbons et al., 1981). In female *T. scripta* a positive correlation exists between adult body size and clutch size (Gibbons, 1970a) and therefore a delay in sexual maturity, resulting in a larger body, would increase fecundity (Brooks et al., 1992).

Research on the growth of freshwater turtles has failed to identify clear trends in turtle growth with universal application among species. However, it has shown that turtle growth varies between species, populations and sexes. Therefore, this study was designed to examine growth, maturation and adult body sizes in two sub-populations of *T. scripta* on the Upper-Coastal Plain of South Carolina, an area with no record in the literature of previous chelonian studies. Previous studies examining growth and adult body size between different aquatic environments have done so by comparing two or more populations of a particular species (e.g., Iverson, 1985; Rowe, 1997). Here we were able to compare growth, maturity and body size between two sub-populations of *T. scripta* based on water temperature, food availability and selection pressures, while limiting the genetic variability between populations. It was expected that the growth rates and adult body sizes of males would be higher in the larger water body (Iverson, 1985); therefore,

male turtles in Dargans' Pond were expected to mature at a larger body size than those in the smaller ponds at PDREC. Based on the positive correlation between body size and clutch size in female *T. scripta* (Gibbons, 1970a), it was expected that female growth and body size would not be different between the two study areas.

Methods

Turtles were trapped using 1.2 m x .9 m hoop-nets with 2.5 cm nylon mesh, baited with canned cat food. Cans were punctured and hung from the center of the trap so that the bait hung between the entrance and the bottom of the trap. Traps were set parallel to the shore for a 24 hour period. Trapping was conducted in Dargans' Pond during the summers of 2002, 2004, 2005 and 2006 and in the six smaller ponds during the summer of 2006. In Dargans' Pond turtles were trapped at 8 sites for a total of 279 trap nights and at 6 sites in the small ponds for 120 trap nights.

Once captured, turtles were identified to species, sexed and weighed using an electronic balance (to the nearest g). Maximum straight-line plastron and carapace length and carapace width were measured using calipers (to the nearest mm) and individual turtles were marked. Individual turtles were distinguished using AVID® PIT (Passive Integrated Transponder) tags. Once measurements were taken, turtles were scanned with an AVID® PIT tag reader in order to determine if they have been previously captured. New captures were then marked by using a syringe to insert a PIT tag into the left inner thigh between the skin and muscle tissue. Individuals weighing less than 100g were marked, following Cagle (1939), by notching the marginal scutes. As individual turtles

were processed they were immediately released at the trap site from which they were captured.

The display of secondary sexual characteristics was used to identify maturity in turtles. In Dargans' Pond and PDREC ponds, the 10 smallest (plastron length) male *Trachemys scripta* displaying secondary sexual characteristics were averaged in order to determine the mean size at maturity. Turtles captured below the mean plastron length and not showing sex specific traits were classified as juveniles.

Growth was estimated from the difference in the maximum plastron length at first and last capture. The growth rate was then calculated in millimeters per year based on the number of days between captures. Each turtle was included in the analysis once in order to avoid the effect of individual variation in growth (Frazier et al., 1991). Turtles with less than 10 days between captures were omitted. The data analyses were performed using SAS, version 9.1.3 (2000-2004). Significance was assumed at P-value < 0.05. The difference in the mean plastron length of male and female turtles between Dargans' Pond and the PDREC ponds was compared using a t-test. A correlation analysis was used to examine the relationship between growth rate and body mass, plastron length, carapace length, and carapace width. Analysis of covariance (ANCOVA) was used to compare the growth rate between sexes in Dargans' Pond and PDREC ponds and to compare growth rate of males and females between Dargans' Pond and PDREC ponds.

Results

The 4 years of the study yielded 802 turtle captures made up of 565 individuals. In Dargans' Pond there were 622 captures of 413 turtles made up of 4 species, being

Kinosternon subrubrum, *Psuedemys concinna*, *Sternotherus odoratus* and *Trachemys scripta*. In the small ponds at PDREC there were 180 captures of 152 turtles made up of 6 species, being *Chelydra serpentina*, *Kinosternon baurii*, *K. subrubrum*, *P. concinna*, *S. odoratus* and *T. scripta*.

In Dargans' Pond, the mean plastron length at which *T. scripta* males were found to mature was 102.6 mm (range: 89-112) and in PDREC ponds, males matured at a mean plastron length of 102.1 mm (range: 92-116), which were not significantly different ($t = 0.13$, $df = 18$, $P = 0.90$). The mean plastron length of male *T. scripta* in Dargans' Pond (mean = 154.1 mm, $SD = 26.1$, $N = 195$) was significantly higher ($t = 2.59$, $df = 259$, $P < 0.01$) than those in PDREC ponds (mean = 144.8 mm, $SD = 22.3$, $N = 66$). The mean plastron length of female *T. scripta* in Dargans' Pond (mean = 193.7 mm, $SD = 37.6$, $N = 197$) was also significantly higher ($t = 1.65$, $df = 242$, $P < 0.05$) than those in PDREC ponds (mean = 183.8 mm, $SD = 33.4$, $N = 47$).

Growth data were collected from 4 species, being *K. subrubrum*, *P. concinna*, *S. odoratus* and *T. scripta*, although, *T. scripta* was the only species recaptured enough to provide a representative sample of growth between sexes and plastron lengths. In Dargans' Pond, growth rates were calculated for 59 individuals of *T. scripta*, being 27 males, 29 females and 3 juveniles. The mean number of days between first and last capture for the 59 *T. scripta* from Dargans' Pond was 443 days. In PDREC ponds, growth rates were calculated for 20 individuals of *T. scripta*, being 9 males, 10 females and 1 juvenile. The mean number of days between first and last capture for the 20 *T. scripta* from PDREC ponds was 48 days.

The correlation analyses revealed a significant ($P < 0.05$) negative, linear relationship between the growth rate of *T. scripta* and body mass, plastron length, carapace length and carapace width for males and females in Dargans' Pond and males and females in the smaller PDREC ponds (Figure 2.1)

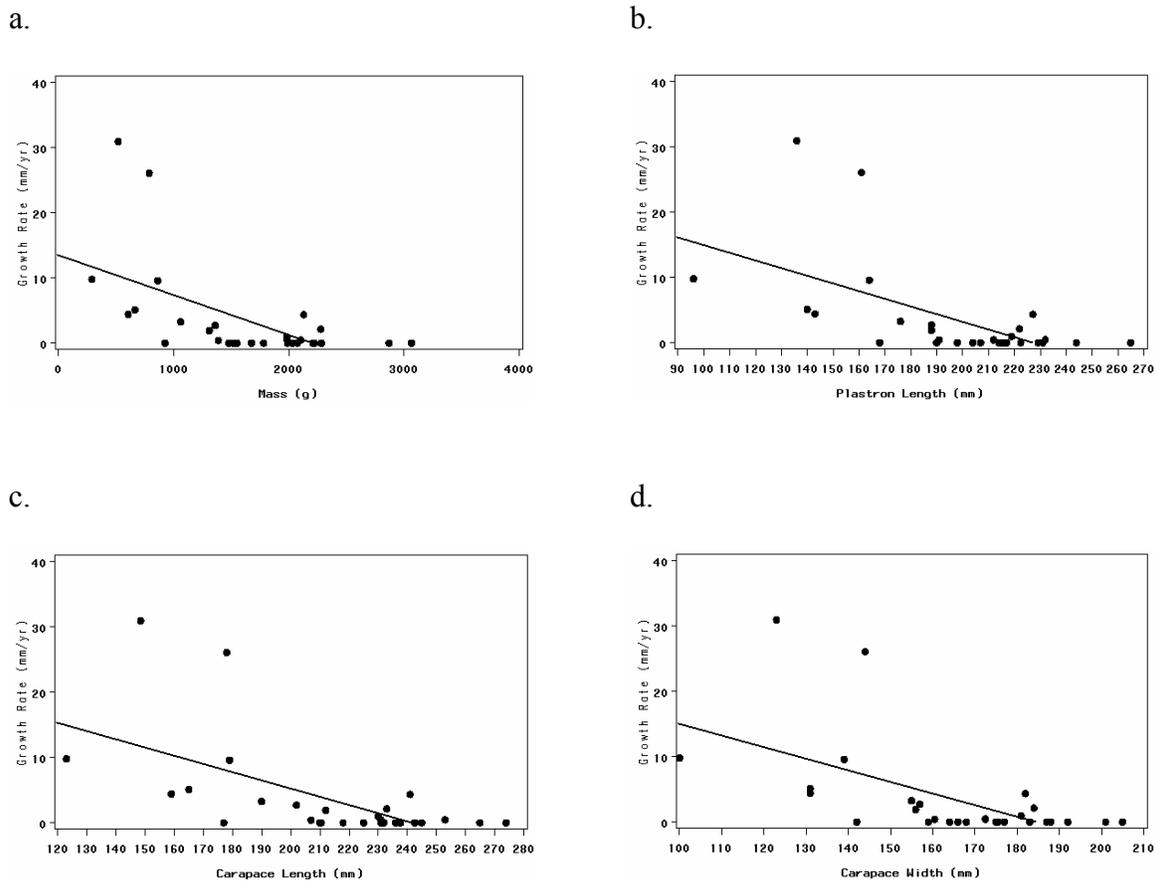


Figure 2.1a-d. The resulting graphs from the correlation analyses comparing the growth rate (mm/yr) of *Trachemys scripta* females in Dargans' Pond to (a) body mass (g), (b) plastron length (mm), (c) carapace length (mm), and (d) carapace width (mm).

In Dargans' Pond the growth rates of *T. scripta* males at plastron lengths of 100, 120, 140, 160 and 180 mm were found to be 7.84, 6.08, 4.32, 2.55 and 0.79 mm/yr. At plastron lengths of 100, 120, 140, 160, 180 and 200 mm, female *T. scripta* in Dargans' Pond were found to experience growth rates of 14.97, 12.61, 10.26, 7.9, 5.54 and 3.19 mm/yr. In Dargans' Pond, the growth rates of female *T. scripta* were significantly higher than those of males at plastron lengths 100 ($P < 0.05$), 120 ($P < 0.05$), 140 ($P < 0.01$), 160 ($P < 0.01$), 180 ($P < 0.05$) and 200 mm ($P < 0.05$) (Figure 2.2).

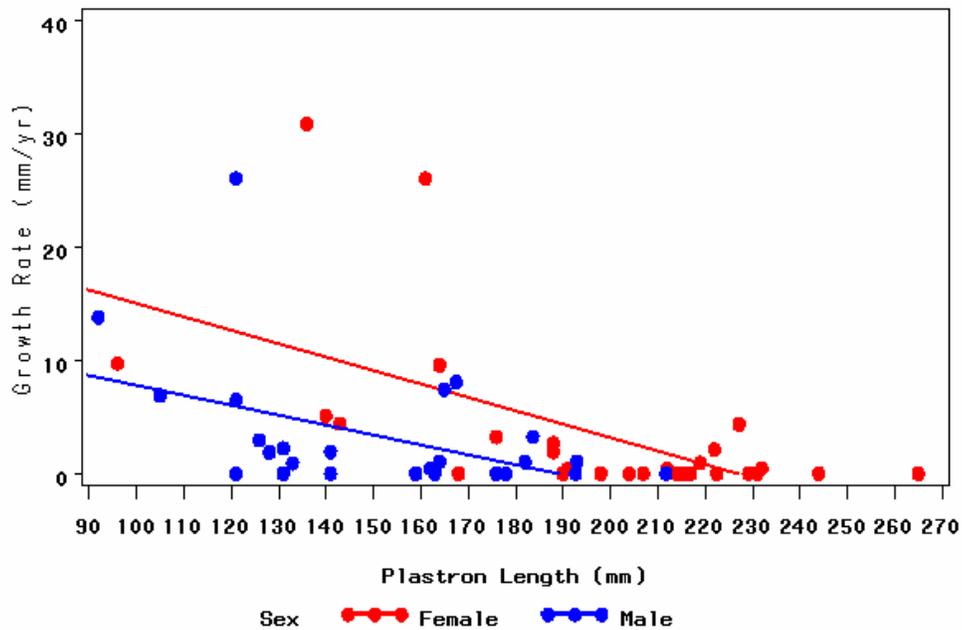


Figure 2.2. A linear model from the ANCOVA, illustrating the growth rate (mm/yr) and plastron lengths (mm) of *Trachemys scripta* captured in Dargans' Pond.

In PDREC ponds, the growth rate of male *T. scripta* at plastron lengths of 100, 120, 140 and 160 mm were found to be 24.9, 15.53, 8.16 and 0.10 mm/yr. At plastron lengths of 100, 120, 140, 160, 180 and 200 mm female *T. scripta* in PDREC ponds were found to experience growth rates of 36.2, 29.2, 23.71, 17.47, 11.22 and 4.98 mm/yr. At a plastron length of 100 mm, the growth rates of female *T. scripta* in PDREC ponds was significantly higher than those of males ($P < 0.05$). At a plastron length of 120 mm, male and female *T. scripta* in PDREC ponds displayed no significant difference ($P = 0.15$) in growth rate, while female growth rates were significantly higher than those of males at 140 ($P < 0.01$), 160 ($P < 0.01$), 180 ($P < 0.01$) and 200 mm ($P < 0.01$) (Figure 2.3).

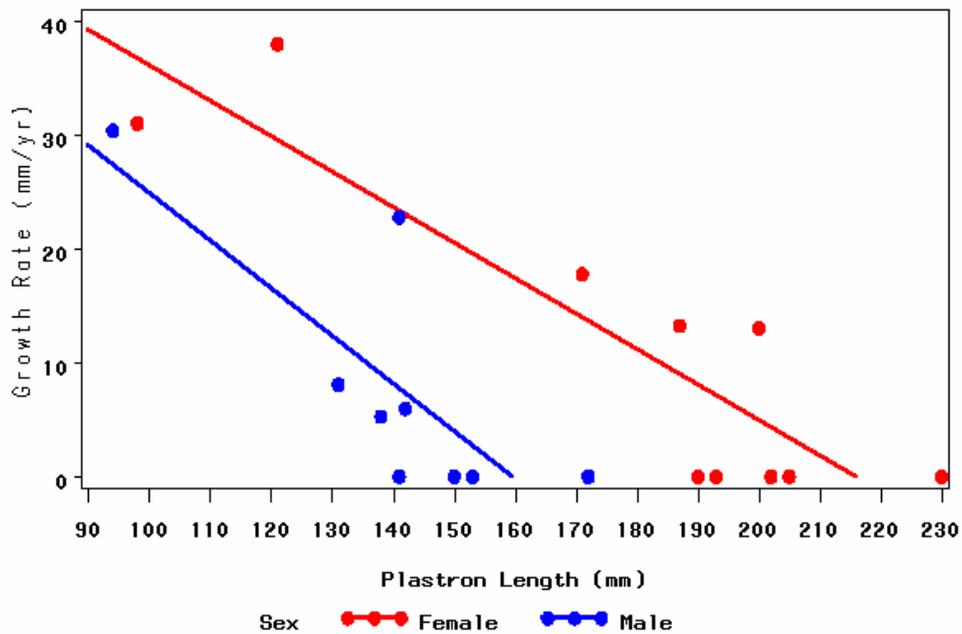


Figure 2.3. A linear model from the ANCOVA, illustrating the growth rate (mm/yr) and plastron lengths (mm) of *Trachemys scripta* captured in the six small ponds at PDREC.

The growth rates of *T. scripta* males in the PDREC ponds was significantly higher than those in Dargans' Pond at plastron lengths 100 ($P < 0.01$), 120 ($P < 0.01$), and 140 mm ($P < 0.01$). At a plastron length of 160 mm, the growth rate of male *T. scripta* did not differ ($P = 0.17$) between the two areas. The growth rates of *T. scripta* males in Dargans' Pond was significantly higher ($P < 0.01$) than those in PDREC ponds at a plastron length of 180 mm (Figure 2.4).

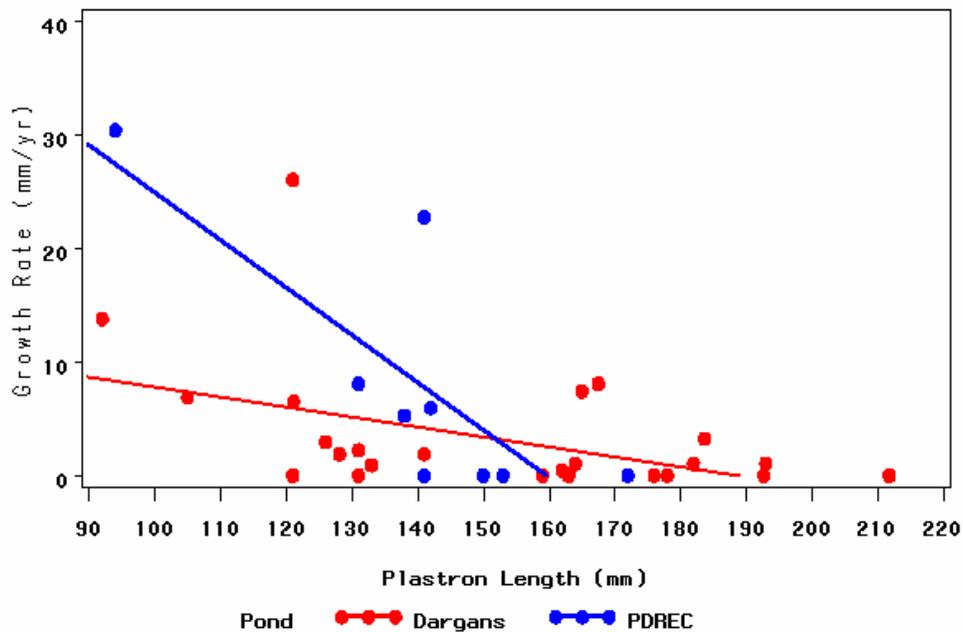


Figure 2.4. A comparison of the growth rates (mm/yr) of male *Trachemys scripta* between Dargans' Pond and the PDREC ponds.

The growth rate of *T. scripta* females in PDREC ponds was significantly higher than those in Dargans' Pond at plastron lengths 100 ($P < 0.01$), 120 ($P < 0.01$), 140 ($P <$

0.01), 160 ($P < 0.01$) and 180 mm ($P < 0.01$). At a plastron length of 200 mm, the growth rate of female *T. scripta* did not differ ($P = 0.24$) between the two areas (Figure 2.5).

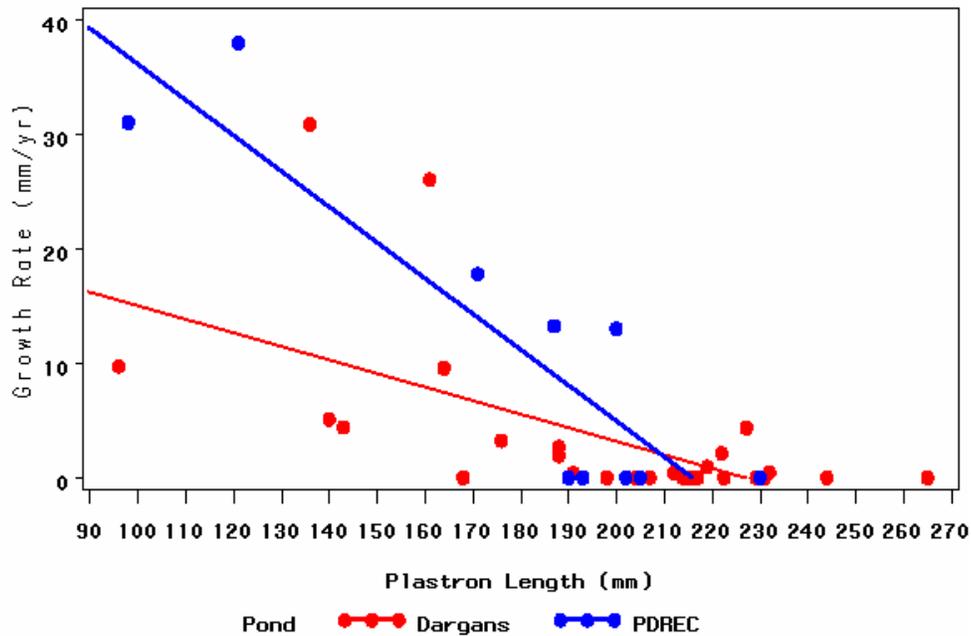


Figure 2.5. A comparison of the growth rates of female *Trachemys scripta* between Dargans' Pond and the PDREC ponds.

Discussion

It has been well documented that *Trachemys scripta* males reach maturity at a much smaller size than do females (Dunham, 1978; Gibbons and Lovich, 1990). Although, the exact size at which maturity is reached varies considerably between individuals and populations (Gibbons et al., 1981). In both Dargans' Pond and PDREC ponds, male *T. scripta* were found to mature at a mean plastron length of 102 mm.

Similarly, Gibbons and Greene (1990) noted, that in two populations of *T. scripta* at the Savannah River Plant, near Aiken, South Carolina, males began to attain maturity at a plastron length of 100 mm. This adheres to the assumption that male *T. scripta* mature at a fixed size rather than a fixed age (Gibbons et al., 1981). Because female turtles were not palpated for eggs during the study it was not possible to determine the size at which they mature sexually. Generally, female *T. scripta* have been found to attain maturity around 160 mm plastron length (Gibbons and Greene, 1990). Although other studies have shown female *T. scripta* to mature at a fixed age rather than a fixed size (Gibbons et al., 1981). Here, *T. scripta* captured above 102 mm, that lacked the presence of male secondary sexual characteristics, elongated foreclaws and precloacal tail, were considered females. Although, this technique does not account for maturity in females, it enabled us to distinguish between juveniles and immature females.

Early research on freshwater chelonians identified growth to be highest during the juvenile stage, while steadily decreasing as turtles increased in size (Cagle, 1946). In this study the correlation analyses identified a significant, negative relationship between growth rate and body size. Therefore, growth in this population of *T. scripta* follows a pattern similar to those encountered in previous studies of freshwater turtles.

In *T. scripta*, growth rates during the juvenile stage do not vary between the sexes (Dunham and Gibbons, 1990; Wallin and Gibbons, 1990). Therefore, it was expected that male and female growth rates would not diverge until turtles had reached a plastron length of 102 mm. Yet, females in both Dargans' Pond and PDREC ponds were found to have significantly higher growth rates than males at a 100 mm plastron length. In

Dargans' Pond, at a plastron length of 100 mm, female *T. scripta* grew 14.97 mm/yr, where males grew 7.84 mm/yr. In PDREC ponds, at a plastron length of 100 mm, *T. scripta* females experienced a growth rate of 36.2 mm/yr, where males experienced a growth rate of 24.9 mm/yr. This suggests that the males are either mature or are beginning to mature around 100 mm plastron length, which would explain their lower growth rates. This is consistent with the estimated maturation size of 102 mm.

Dunham (1978) stated that in sexually dimorphic species, such as *T. scripta*, where females are larger than males, the absolute growth rate of females must be consistently higher than those of males. Similarly, *T. scripta* females in both Dargans' Pond and PDREC ponds experienced significantly higher growth rates than males of similar size. Although, in PDREC ponds, at 120 mm plastron length there was no difference in the growth rate between males and females.

Turtle growth is highly variable between similar sized individuals belonging to the same sex and population (Cagle, 1946; Bury, 1979; Andrews, 1982; Dunham and Gibbons, 1990). This was evident in the population of *T. scripta* inhabiting both Dargans' Pond and the smaller PDREC ponds. In Dargans' Pond, two females captured and recaptured in the same month and year, May 2004 and August 2006, with similar plastron lengths, of 188 and 190 mm, had very different growth patterns. With over 800 days between first and last capture, the 188 mm turtle grew 6 mm, while the 190 mm turtle showed no growth. In the PDREC ponds, two males captured and recaptured in the same month and year, May 2006 and July 2006, both with plastron lengths of 141 mm, grew 3 mm, while the other showed no growth. This type of variability makes turtle growth

difficult to quantify without the aid of a very large sample size. This is, in part, due to the numerous factors that have been shown to influence growth in freshwater turtles.

In turtles, as with most organisms, food availability is one of many factors that influence growth (Mahmoud, 1969; MacCulloch and Secoy, 1985). Iverson (1985) suggested that resource availability may be highest in large aquatic habitats. Therefore, all else equal, both male and female *T. scripta* in Dargans' Pond should experience higher growth rates than those in PDREC ponds. Yet, at 100, 120 and 140 mm plastron lengths, males in PDREC ponds had significantly higher growth rates than those in Dargans' Pond and at 100, 120, 140, 160 and 180 mm plastron lengths, females in PDREC ponds experienced significantly higher growth rates than those in Dargans' Pond (Table 2.3). This may be attributed to the difference in the relative size of the two ponds. The smaller ponds at PDREC have a larger edge to area ratio than Dargans' Pond, which creates a larger margin of shallow water around the pond allowing more aquatic vegetation per unit area. Therefore, food availability may be higher in the smaller ponds, because the diet of adult *T. scripta* is composed largely of aquatic vegetation (Parmenter, 1980; Parmenter and Avery, 1990), explaining why, growth rates would be highest in the smaller PDREC ponds.

Table 2.1. The calculated growth rates of male and female *Trachemys scripta* from Dargans' Pond and PDREC Ponds.

Plastron Length (mm)	Growth Rate (mm/yr.)			
	Dargans' Pond		PDREC ponds	
	Male	Female	Male	Female
100	7.84	14.97	24.9	36.2
120	6.08	12.61	16.53	29.2
140	4.32	10.26	8.16	23.71
160	2.55	7.9	0.1	17.47
180	0.79	5.54		11.22
200		3.19		4.98

Growth in freshwater turtles has also been found to be dependent upon water temperature. Turtles inhabiting thermally influenced ponds have been found to feed on high-quality food items (Parmenter, 1980), have increased digestive activity (Kepeniz and McManus, 1974; Parmenter, 1980; Frazer et al., 1991), accelerated growth rates (Gibbons, 1970b; Parmenter, 1980), and increased body sizes (Gibbons, 1970b; Parmenter, 1980), when compared to turtles inhabiting natural habitats. Although the ponds in this study are not thermally influenced by nuclear reactors, the small size and shallow water of the six PDREC ponds result in higher summer water temperatures when compared to the larger Dargans' Pond. Parmenter (1980) recognized that slight differences in water temperature are significant when considering the physiological impact on turtle's feeding ecology. In thermally influenced habitats, *T. scripta* has been found to grow rapidly after maturity has been reached (Parmenter, 1980). This may explain why there was no difference between the growth rates of males and females with 120 mm plastron lengths in PDREC ponds, meaning that sexually mature males grew as

rapidly as immature females at a 120 mm plastron length. Therefore, the accelerated growth rates of *T. scripta* inhabiting the smaller PDREC ponds suggests that water temperature may play a pivotal role in turtle growth.

As expected, both male and female *T. scripta* inhabiting Dargans' Pond had significantly larger mean plastron lengths than those in PDREC ponds. Although, the original hypothesis was made assuming that growth would also be higher in Dargans' Pond. Werner and Gilliam (1984) assumed growth to be the primary factor affecting adult body size in species of size-structured populations. If this were the case, the mean plastron length for male and female *T. scripta* would have been larger in the PDREC ponds because this is where growth was the highest. Therefore, it could be assumed that adult body sizes in these two sub-populations are influenced by other factors, such as selection pressures.

Both natural and sexual selection are thought to influence body size in turtles. Many freshwater turtle species experience sexual dimorphism in size and age at maturity, suggesting a dual adaptive strategy between the sexes (Tinkle, 1961; Gibbons et al., 1981). In female *T. scripta* a positive correlation exists between body size and clutch size (Gibbons, 1970a) and therefore a delay in sexual maturity, resulting in a larger body, would increase fecundity (Brooks et al., 1992). In male *T. scripta*, early-maturation allows individuals to enter the breeding population earlier in life allowing energy to be reallocated for mate searching and courtship behavior (Congdon et al., 1982). Although, if a small size alone was advantageous, continued growth after maturity would be maladaptive, therefore, small individuals may not be competitive in the breeding

population, less likely to avoid predation or unable to effectively cope with environmental aspects (Gibbons and Lovich, 1990). Therefore natural selection and sexual selection are opposing forces influencing body size.

In general, freshwater turtles experience considerable mortality, primarily through predation, in eggs, hatchlings and small juveniles, with relatively low and constant mortality at the large juvenile and adult stages (Gibbons and Semlitsch, 1982; Iverson, 1991). Delayed-maturity would act to increase the adult body size, spending more time in the juvenile stage, characterized by rapid growth, which would increase survival.

Gibbons (1970a) suggested that turtles may need to reach a minimum size before they are able to escape predators, such as largemouth bass (*Micropterus salmoides*) and alligators (*Alligator mississippiensis*). Although, later studies found that largemouth bass do not feed on live turtle hatchlings (Semlitsch and Gibbons, 1989; Briston and Gutzke, 1993) and alligators are seldom encountered at the study site. Therefore, it could be assumed that natural selection pressures, via predation, are not responsible for the difference in body size between the two areas.

Gibbons and Lovich (1990) stated that larger turtles are more successful in long tracks overland and aquatic habitats. This may explain why *T. scripta* in Dargans' Pond have a larger mean body size than those in PDREC ponds. The growth rate of *T. scripta* in PDREC ponds appears to decrease rapidly as plastron length increases when compared to Dargans' Pond. In female *T. scripta*, once turtles reached a plastron length of 200 mm there was no difference in the growth rate between the two areas. In male *T. scripta* there was no difference in the growth rates between Dargans' Pond and PDREC ponds at 160

mm plastron length and once turtles reached 180 mm plastron length, growth rates were highest in Dargans' Pond. This could explain why both male and female turtles in Dargans' Pond had a larger mean plastron length. In Dargans' Pond turtles appear to continue to grow at a relatively stable, decreasing rate, where growth in PDREC ponds decreases rapidly with increasing size. Therefore, *T. scripta* inhabiting Dargans' Pond would eventually achieve a larger size than those in PDREC ponds. This would be a product of natural selection based upon the turtle's environment. The larger size of Dargans' Pond allows turtles to travel farther; therefore, a larger body size would be advantageous in this habitat, suggesting body size is influenced primarily through natural selection. In the smaller PDREC ponds, turtles only need to move short distances relative to those in Dargans' Pond, therefore a smaller body size would be advantageous in this habitat, suggesting body size is influenced primarily through sexual selection.

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CHAPTER THREE

FRESHWATER CHELONIAN ECTOPARASITE INFESTATION BY THE LEECH *PLACOBDELLA PARASITICA*

Abstract

The occurrence of leeches (Hirudinea) on freshwater turtle species was studied during July and August, 2006 at Pee Dee Research and Education Center (PDREC) on the Upper Coastal Plain of South Carolina. Turtles were trapped in Dargans' Pond, about 150 acres in size and in a string of 6 small ponds at PDREC, all less than 4 acres in size, using hoop-nets. A total of 30 turtles were randomly selected from each of the two areas and examined for leeches. The number of leeches on each turtle were counted and collected. All five turtle species captured during the study, *Kinosternon baurii*, *Kinosternon subrubrum*, *Pseudemys concinna*, *Sternotherus odoratus* and *Trachemys scripta*, were parasitized by leeches. *T. scripta* made up the majority of turtle captures in both Dargans' Pond (97%) and in the smaller ponds at PDREC (87%). The only species of leech found parasitizing turtles was *Placobdella parasitica*. There was no significant difference in the percentage of *T. scripta* infested with leeches, the mean number of leeches per turtle or the mean number of leeches per parasitized turtle between Dargans' Pond and the smaller ponds at PDREC. There was no significant difference between male and female *T. scripta* in the percentage of turtles infested with leeches, the mean number of leeches per turtle or the mean number of leeches per parasitized turtle. There was a significant positive relationship between the number of leeches parasitizing *T. scripta* and body mass, plastron length, carapace length and carapace width. Aspects of the

relationship between freshwater chelonians and the leech *Placobdella parasitica* are presented.

Introduction

The genus *Placobdella* represents 50% of the North American leeches known to parasitize turtles (Graham et al., 1997) with 3 species, in particular, being common ectoparasites of freshwater chelonians: *Placobdella papillifera*, *Placobdella parasitica*, and *Placobdella ornata* (Siddall and Gaffney, 2004). Of these three species, *P. parasitica* has been documented as the most abundant species found on turtles (Ernst, 1971; Koffler et al., 1978; MacCulloch, 1981; Brooks et al., 1990; Saumure and Livingston, 1994; Graham et al., 1997). In fact, *P. parasitica* has been documented parasitizing *Chelydra serpentina* (Maloney and Chandler, 1976; Dodd, 1988a; Brooks et al., 1990; Brown et al., 1994), *Chrysemys picta* (Ernst, 1971; Siddall and Gaffney, 2004), *Chrysemys picta belli* (MacCulloch, 1981), *Clemmys insculpta* (Koffler et al., 1978; Farrell and Graham, 1991), *Clemmys guttata* (Ernst, 1976), *Graptemys geographica* (Graham et al., 1997), *Graptemys pulchra* (Dodd, 1988a), *Pseudemys concinna* (Dodd 1988a), *Sternotherus depressus* (Dodd, 1988a), *Sternotherus odoratus* (Ernst 1986; Dodd 1988a), *Trachemys scripta* (Maloney and Chandler, 1976; Dodd, 1988a) and *Trionyx spiniferus* (Dodd, 1988a).

Several early studies focused on documenting the seasonal pattern of leech infestation on freshwater turtles. This was first studied in Lancaster County, Pennsylvania by Ernst (1971), who found leech (*P. parasitica*) infestations on the turtle *C. picta* to peak in May while being absent from October through February. Similarly, Ernst (1976)

found leech infestation on the turtle *C. guttata* to peak in early summer. Koffler et al. (1978) used a similar design to document the seasonal occurrence of leeches (*P. parasitica*) on the turtle *C. insculpta* in Ulster County, New York and Morris County, New Jersey, where they found leech infestation to peak in October, November and April. They attributed this difference to the behavior of host, *C. insculpta*, which spends much of its time away from water during the warmer months (Koffler et al., 1978).

The direct effect of leech parasitism on freshwater turtles is not known. Brown et al. (1994) predicted that reproductive success of female *C. serpentina* would be reduced as a result of leech (*P. parasitica*) infestation but found that females with more leeches in 1990 produced larger clutches the following year than those with little to no leech infestation. In another study, Dodd (1988b) found that diseased *S. depressus* were not parasitized by leeches whereas healthy turtles were. This was attributed to the tendency of diseased turtles to bask more often than healthy turtles which would cause ectoparasites to drop off the turtle to avoid desiccation (Dodd, 1988b). It was also suggested that this could be due to resource quality, where diseased turtles were probably poorer in resource quality than healthy turtles (Dodd, 1988b).

Other studies have taken a more general approach in studying the relationship between freshwater chelonians and the leech *P. parasitica* by comparing infestation between male and female turtles. The mean leech abundance on the turtle *G. geographica*, in Vermont, was found to be significantly higher on females than on males (Graham et al., 1997). In contrast, Brooks et al. (1990) found that the mean leech abundance on the turtle *C. serpentina*, in Southeastern Ontario, was not different between

the sexes. In the same study they found that the number of leeches on *C. serpentina* was not correlated with the turtles mass (Brooks et al., 1990).

The leech *P. parasitica* is abundant and widespread throughout the Southeast, although very little research exists on the relationship between this leech and freshwater turtles of the region. Therefore this study was designed to investigate the relationship between freshwater turtles and *P. parasitica*. The focus of the study was to examine leech parasitism among freshwater turtles between a large water body, Dargans' Pond, and a smaller water body, PDREC ponds, with similar habitat features. It was expected, that leech parasitism would be higher in the smaller ponds because turtles would be less mobile than those in the larger pond and therefore more susceptible to leech parasitism. Another objective was to investigate leech abundance between sexes. It was estimated that leech parasitism among turtle species exhibiting sexual size dimorphism, such as *T. scripta*, where females are known to be larger than males (Gibbons and Greene, 1990; Gibbons and Lovich, 1990), would be higher in females. The final objective was to examine the relationship between the body mass and size of a turtle with the number of leeches. It was expected that larger turtles would have a higher number of leeches parasitizing it than that of a smaller turtle.

Methods

Turtles were trapped using 1.2m x .91m hoop-nets with 9.67cm² nylon mesh, baited with canned cat food. Cans were punctured and hung from the center of the trap so that the bait hung between the entrance and the bottom of the trap. Traps were set parallel to the shore for a 24 hour period. Turtles were trapped in both Dargans' Pond and the

smaller PDREC ponds during July and August 2006. In Dargans' Pond 8 traps were set daily during the study period at 8 trap sites. In the smaller PDREC ponds 1 trap was used per pond at 2 trap sites. A total of 30 turtles were randomly selected from each of the two areas.

Once captured, turtles were identified to species, sexed, and weighed using an electronic balance (to the nearest g). Maximum straight-line plastron and carapace length and carapace width were measured using calipers (to the nearest mm) and individual turtles were marked. Individual turtles were distinguished using AVID® PIT (Passive Integrated Transponder) tags. Once measurements were taken, turtles were scanned with an AVID® PIT tag reader in order to determine if they have been previously captured. New captures were then marked by using a syringe to insert a PIT tag into the left inner thigh between the skin and muscle tissue. Individuals weighing less than 100g were marked, following Cagle (1939), by notching the marginal scutes. The leeches on each turtle were counted and collected using forceps and placed in a vial of water. As individual turtles were processed they were immediately released at the trap site from which they were captured.

The leeches were then transported to the lab where they were narcotized in a solution of water and ethanol. A stereo microscope was used to identify the leeches to species based on the taxonomic key of Klemm (1995).

The data analyses were performed using SAS, version 9.1.3 (2000-2004). Significance was assumed at $P < 0.05$. A chi-square test was used to test the difference in the percentage of turtles infested with leeches between Dargans' Pond and PDREC

ponds. Fisher's Exact Test was used to identify differences in the percentage of male and female turtles infested with leeches in Dargans' Pond and PDREC ponds. It was also used to test the difference in the percentage of male and female turtles infested with leeches between Dargans' Pond and PDREC ponds. A t-test was used to test the difference in the mean number of leeches per turtle between Dargans' Pond and PDREC ponds, males and females in Dargans' Pond, males and females in PDREC ponds, males in Dargans' Pond and males in PDREC ponds, and females in Dargans' Pond and females in PDREC ponds. A t-test was also used to identify differences in the mean number of leeches per parasitized turtle (≥ 1 leech) between Dargans' Pond and PDREC ponds, males and females in Dargans' Pond, males and females in PDREC ponds, males in Dargans' Pond and males in PDREC ponds, and females in Dargans' Pond and females in PDREC ponds. The correlation procedure was used in SAS to identify significant linear relationships between the number of leeches per turtle and the turtle's body mass, plastron length, carapace length, and carapace width.

Results

The 30 turtles sampled from Dargans' Pond were made up of 2 species being 1 *Kinosternon subrubrum* (3.3%) and 29 *Trachemys scripta* (96.7%), while the 30 turtles sampled from the smaller PDREC ponds were made up of 4 species, being 1 *Kinosternon baurii* (3.3%), 1 *Pseudemys concinna* (3.3%), 2 *Sternotherus odoratus* (6.7%), and 26 *T. scripta* (86.7%) (Table 3.1).

Table 3.1. The species and sex of the 30 turtles randomly selected for the leech survey.

Species	Dargans'			PDREC			Total
	Male	Female	Juvenile	Male	Female	Juvenile	
<i>Kinosternon baurii</i>	0	0	0	1	0	0	1
<i>Kinosternon subrubrum</i>	1	0	0	0	0	0	1
<i>Pseudemys concinna</i>	0	0	0	0	1	0	1
<i>Sternotherus odoratus</i>	0	0	0	1	1	0	2
<i>Trachemys scripta</i>	17	10	2	13	11	2	55

A total of 145 leeches were removed from the turtles captured in the PDREC ponds. All were *P. parasitica*. Of the 26 *T. scripta* captured in PDREC ponds, 22 contained at least 1 leech (84.6%) with a mean of 4.3 (SD = 5.28) leeches per turtle. The 22 *T. scripta* parasitized had a mean of 5.1 (SD = 5.37) leeches. Of the 13 male *T. scripta* captured in the PDREC ponds, 11 contained at least 1 leech (84.6%) with a mean of 4.3 (SD = 5.82) leeches per turtle. The 11 male *T. scripta* parasitized had a mean of 5.1 (SD = 6.02) leeches. Of the 11 female *T. scripta* captured in the PDREC ponds, 10 contained at least 1 leech (90.9%) with a mean of 4.9 (SD = 5.15) leeches per turtle. The 10 female *T. scripta* parasitized had a mean of 5.4 (SD = 5.15) leeches.

Fisher's Exact Test showed that there was no difference between the percentage of male and female *T. scripta* parasitized by leeches (P = 0.23) in PDREC ponds. A t-test found that there was no difference in the mean number of leeches between male and female *T. scripta* in PDREC ponds (t = 0.27, df = 22, P = 0.79). Of the *T. scripta* parasitized by leeches, there was no difference in the mean number of leeches per male compared to that of females (t = 0.13, df = 19, P = 0.90).

A total of 98 leeches were removed from the turtles captured in Dargans' Pond. All of which were *P. parasitica*. Of the 29 *T. scripta* captured in Dargans' Pond, 18 contained at least 1 leech (62.1%) with a mean of 3.3 (SD = 6.93) leeches per turtle. The 18 *T. scripta* parasitized had a mean of 5.3 (SD = 5.65) leeches per turtle. Of the 17 male *T. scripta* captured in Dargans' Pond, 10 contained at least 1 leech (58.8%) with a mean of 2.6 (SD = 3.14) leeches per turtle. The 10 male *T. scripta* parasitized had a mean of 4.4 (SD = 2.95) leeches. Of the 10 female *T. scripta* captured in Dargans' Pond, 8 contained at least 1 leech (80%) with a mean of 5.2 (SD = 7.54) leeches per turtle. The 8 female *T. scripta* parasitized had a mean of 6.5 leeches (SD = 7.96).

Fisher's Exact Test showed that there was no difference between the percentage of male and female *T. scripta* parasitized by leeches (P = 0.41) in Dargans' Pond. A t-test found that there was no difference in the mean number of leeches between male and female *T. scripta* in PDREC ponds (t = 1.04, df = 10.9, P = 0.32). Of the *T. scripta* parasitized by leeches, there was no difference in the mean number of leeches per male compared to that of females (t = 0.71, df = 8.54, P = 0.50).

A chi-square test of the percentage of *T. scripta* infested with leeches between Dargans' Pond (62.1%) and the smaller PDREC ponds (84.6%) showed no significant difference ($\chi^2 = 3.51$, P = 0.06). Similarly, the mean number of leeches per *T. scripta* between Dargans' Pond (mean = 3.31, SD = 5.13, N = 29) and PDREC ponds (mean = 4.35, SD = 5.28, N = 26) was not found to be significantly different (t = -0.74, df = 53, P = 0.46). Of those parasitized, the mean number of leeches per *T. scripta* in Dargans' Pond

(mean = 5.33, SD = 5.65, N = 18) versus that of PDREC ponds (mean = 5.14, SD = 5.37, N = 22) showed no significant difference ($t = 0.11$, $df = 38$, $P = 0.91$).

The percentage of male *T. scripta* infested with leeches between Dargans' Pond (58.8%) and the smaller PDREC ponds (84.6%), using Fisher's Exact Test, showed no significant difference ($P = 0.23$). Similarly, the mean number of leeches per male *T. scripta* between Dargans' Pond (mean = 2.59, SD = 3.14, N = 17) and PDREC ponds (mean = 4.31, SD = 5.82, N = 13) was not found to be significantly different ($t = -0.96$, $df = 17.3$, $P = 0.35$). Of those parasitized, the mean number of leeches per male *T. scripta* in Dargans' Pond (mean = 4.4, SD = 2.95, N = 10) versus that of PDREC ponds (mean = 5.09, SD = 6.02, N = 11) showed no significant difference ($t = -0.34$, $df = 14.8$, $P = 0.74$).

The percentage of female *T. scripta* infested with leeches between Dargans' Pond (80%) and the smaller PDREC ponds (90.9%), using Fisher's Exact Test, showed no significant difference ($P = 0.59$). Similarly, the mean number of leeches per female *T. scripta* between Dargans' Pond (mean = 5.2, SD = 7.53, N = 10) and PDREC ponds (mean = 4.91, SD = 5.15, N = 11) was not found to be significantly different ($t = 0.10$, $df = 19$, $P = 0.92$). Of those parasitized, the mean number of leeches per female *T. scripta* in Dargans' Pond (mean = 6.5, SD = 7.96, N = 8) versus that of PDREC ponds (mean = 5.4, SD = 5.15, N = 10) showed no significant difference ($t = 0.36$, $df = 16$, $P = 0.73$).

The correlation analyses of the number of leeches per turtle with body mass ($\rho = 0.31$, $P < 0.05$), plastron length ($\rho = 0.34$, $P < 0.01$), carapace length ($\rho = 0.33$, $P < 0.01$) and carapace width ($\rho = 0.35$, $P < 0.01$) all showed a significant positive linear relationship (Figure 3.1a-d).

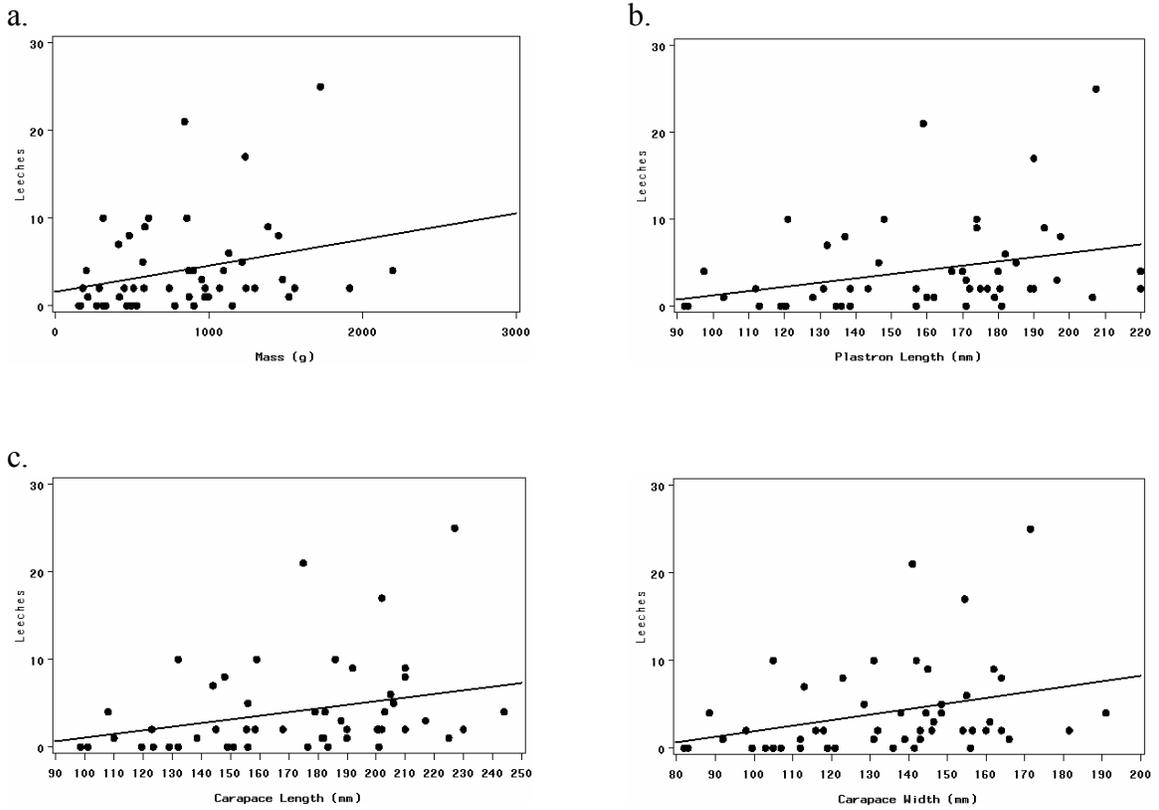


Figure 3.1a-d. The resulting graphs from the correlation analyses comparing the number of *Placobdella parasitica* on *Trachemys scripta* to (a) body mass (g), (b) plastron length (mm), (c) carapace length (mm), and (d) carapace width (mm).

Discussion

All five turtle species encountered during the study were parasitized by *Placobdella parasitica*. Of these, *Kinosternon baurii* had never been documented as host to *P. parasitica*. Most likely, this is due to the lack of existing research on the relationship between freshwater chelonians and *P. parasitica*, which has been documented as a widespread species commonly found on freshwater turtles (Ernst, 1971; Koffler et al., 1978; MacCulloch, 1981; Brooks et al., 1990; Saumure and Livingston, 1994; Klemm, 1995; Graham et al., 1997). Therefore, it is highly unlikely that this leech specializes on a specific group of turtle species. In fact, the turtle species that *P. parasitica* has been documented parasitizing occupy a wide range of habitats and exhibit very different behaviors.

The only turtle species encountered enough for statistical analyses was *Trachemys scripta* which made up 92% of the total captures. This corresponds with the on-going freshwater turtle research at PDREC since 2002, which has documented the dominance of *T. scripta* in these communities. Although a larger sample size may have produced enough individuals of other species to afford statistical analyses.

Although *T. scripta* had been documented as a host to *P. parasitica* (Maloney and Chandler, 1976), the rate of infestation has not been studied. Of the 51 *T. scripta* encountered during the study, 39 (76%) contained at least 1 leech. In Dargans' Pond 62% and in PDREC ponds 85% of the *T. scripta* examined contained at least 1 leech. These parasitism rates are comparable to those found in studies of other freshwater turtle species. Brooks et al. (1990) studied *Chelydra serpentina* in Ontario to find that 84%

were infested with leeches, while MacCulloch (1981) found only 32% of *Chrysemys picta belli* to be parasitized by leeches in Saskatchewan. The rate of leech infestation among turtle species most likely represents differences in behavior and habitat use by each species. Several studies have succeeded in documenting the seasonal patterns of which leech infestation occurs and therefore differences in the rates of leech parasitism on freshwater turtles could reflect the time period in which the study took place.

The original prediction stated leech parasitism on turtles would be higher in the smaller ponds than in the larger pond. It was expected that turtles in the larger pond, Dargans' Pond, would be more mobile based on the availability of open water while the turtles in the smaller, PDREC, ponds would be confined to much smaller areas making them more susceptible to leech parasitism. According to the data taken from *T. scripta* there was no significant difference in the percentage of turtles parasitized, the mean number of leeches per turtle or the mean number of leeches per parasitized turtle between the two areas. Sawyer (1986) found food organisms to be the most important factor in determining the density of *P. parasitica* in a given habitat. Therefore, the population density of *T. scripta* may be the determining factor in the amount of leech parasitism between the two areas. Sawyer (1986) also documented *P. parasitica* remaining attached to its turtle host for extended periods of time, for multiple feedings (Sawyer, 1986). Therefore, once a leech is attached to *T. scripta* its aquatic habitat use should not affect the ability of the leech to parasitize the turtle.

It was estimated that leech parasitism among turtle species exhibiting sexual size dimorphism, such as *T. scripta*, where females are known to be larger than males

(Gibbons and Greene, 1990; Gibbons and Lovich, 1990), would be higher in females.

This is based on the idea that the larger size of females would provide a greater surface area for leech attachment (Dodd, 1988a; Brooks et al., 1990). Although, the results from *T. scripta* indicate that there was no significant difference between the mean number of leeches per turtle and the mean number of leeches per parasitized turtle between the two sexes. Brooks et al. (1990) documented similar results, finding no difference in the number of solitary leeches on male and female *C. serpentina*, a species where males grow larger than females. While another study found *P. parasitica* parasitizing *Graptemys geographica* females significantly more than males (Graham et al., 1997), the most size-dimorphic genera of emydid turtles (Lindeman, 1999).

As predicted, a significantly positive correlation was found to exist between the number of leeches parasitizing *T. scripta* and the body mass, plastron length, carapace length, and carapace width of the turtle. This conflicts with the results regarding leech parasitism among male and female *T. scripta*. Because females are known to grow larger than males (Gibbons and Greene, 1990; Gibbons and Lovich, 1990) they should have had a significantly higher leech load than males, yet there was no significance difference between males and females: in the percentage parasitized, the mean number of leeches per turtle or the mean number of leeches on parasitized turtles. Brown et al. (1994) found no relationship between body mass and leech infestation in female *C. serpentina*. Although, they ranked leech intensity into groups instead of directly counting the number of leeches present on each turtle.

It is clear that *P. parasitica* relies heavily on freshwater chelonians during its life history. The data taken from *T. scripta* suggests that the number of leeches parasitizing a turtle directly correlates to the size of the turtle. Although several studies have attempted to explain aspects of this relationship, the direct effect leeches have on their turtle host is not known. Future research is needed to reveal the complexities of this relationship.

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APPENDICES

Appendix A

Study Site

The study was conducted on the Upper-Coastal Plain of South Carolina at Clemson University's Pee Dee Research and Education Center (PDREC) in Darlington County. The primary focus of research and education at the PDREC is agricultural production and natural resource management. The property encompasses approximately 2300 acres consisting of agricultural fields, forests, and wetlands. More than 14 man-made ponds of varying size occur on the property. The focus area of this project was Dargans' Pond and six adjoining ponds on the PDREC property, all of which are part of the Pee Dee River watershed system (Figure A.1).



Figure A.1. An aerial view of Pee Dee Research and Education center (PDREC) and the area surrounding the PDREC ponds (outlined in yellow) and Dargans' Pond.

The six ponds chosen for the study are thought to have been created sometime in the mid-1900's by the impoundment of existing drainages (Figure A.2). They are separated by a series of man-made dams and are connected hydrologically. Several of the original dams are no longer intact, and therefore water levels fluctuate with beaver (*Castor canadensis*) activity and rainfall.

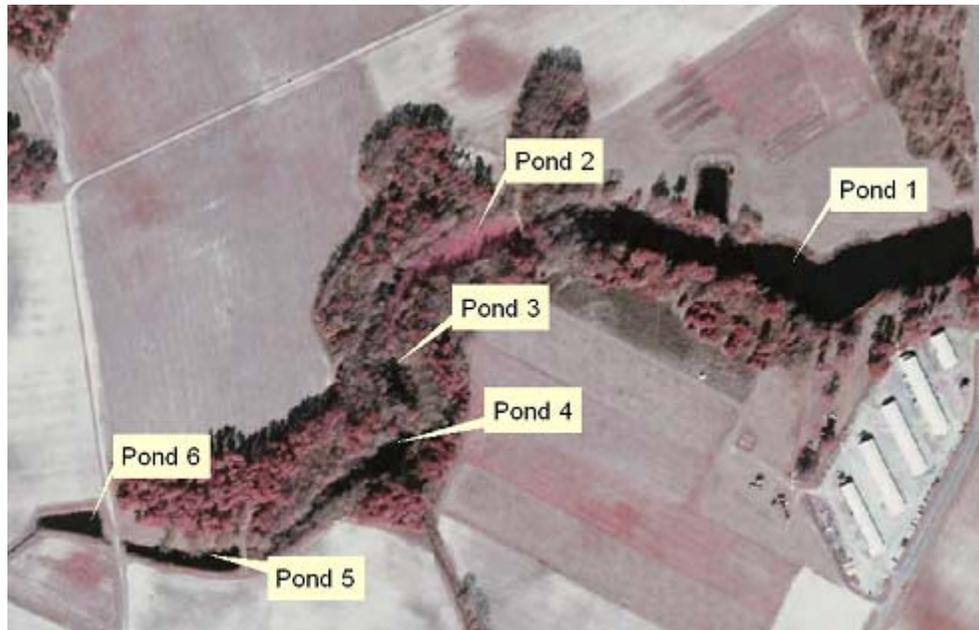


Figure A.2. An aerial view of the six ponds comprising the PDREC ponds described in the study.

The largest of the six ponds, Pond 1, is approximately 5.4 acres in size. Here, the water level is the most stable of the six ponds, being controlled by an overflow pipe. The largest portion of the pond can be characterized as open water, with vegetation in the pond margins being typical of a freshwater marsh. Dominant species of floating vegetation in the open water are being yellow pond lily (*Nephar luteum*) and duckweed (*Lemna* spp.). Dominant species in the moist, shallow margins include alder (*Alnus serulata*), cattail (*Typha latifolia*), rush (*Juncus* spp.), angle-stem primrose (*Ludwigia* spp.), marsh bulrush (*Scirpus cyperinus*) and plumegrass (*Erianthus* spp.). The upper, shallower end of the pond grades into swamp habitat with a canopy dominated by bald cypress (*Taxodium distichum*), red maple (*Acer rubrum*) and black willow (*Salix nigra*).

The pond is surrounded by a riparian buffer, established in 2004, made up, primarily, of alder, cattail, rush, angle-stem primrose, marsh bulrush and plumegrass (*Eriophorum* spp.).

Pond 2 (1.7 acres) is subject to drying during periods of prolonged drought, which last occurred in 2002. The surface of the pond is completely covered by a layer of duckweed throughout the year. The margins of the pond are dominated black willow (*Salix nigra*), buttonbush (*Cephalanthus occidentalis*), elderberry (*Sambucus canadensis*) and seedbox (*Ludwigia alternifolia*). The banks around the pond are forested with the dominant vegetation being river birch (*Betula nigra*), black willow and water oak (*Quercus nigra*).

Pond 3 (0.3 acres) is the smallest. A portion of the original dam has been broken and is now maintained by beaver activity. The water level fluctuates dramatically with beaver activity and/or rainfall. During the study period water levels varied between, approximately 2 meters and 0.25 meters. Vegetation occurs primarily in the margins and is dominated by black willow, elderberry, bald cypress (*Taxodium distichum*), and red maple.

The largest portion of Pond 4 (0.9 acres) is characterized by open water, with the margins dominated by yellow pond lily. The upper, shallower end of the pond grades into a habitat dominated by elderberry, bald cypress, black willow and red maple.

Pond 5 (0.5 acres) and Pond 6 (0.4 acres) are similar in habitat and surroundings. Both are almost completely surrounded by agricultural fields and are sometimes used for irrigation purposes. The margins are dominated by yellow pond lily and the surrounding

vegetation is dominated by of rush, elderberry and buttonbush. Pond 6 directly receives drainage from agricultural and managed timber lands via a small ditch.

The string of six ponds eventually drain into the much larger Dargans' Pond (approximately 150 acres). Dargans' Pond is managed for fisheries, waterfowl, shorebirds and wading birds. It is open to the public for fishing between April 1 and September 30 through a cooperative agreement between Clemson University and the South Carolina Department of Natural Resources. Since 2004, a riser board drainage system has been used to partially draw down the water level in August to stimulate seed germination and the rapid growth of aquatic plants. The pond is then flooded in November to enhance the habitat for migrating waterfowl. The majority of the pond is characterized by open water habitat with the margins being dominated by bald cypress and tupelo gum (*Nyssa aquatica*). This progresses into a shallower, freshwater marsh system with the dominant vegetation being buttonbush and yellow pond lily. Progressing upstream, the freshwater marsh grades into bottomland swamp with a partially closed canopy dominated by bald cypress, tupelo gum, red maple, water oak and sweet gum (*Liquidambar styraciflua*).

Appendix B

Marking Turtles

In this study, turtles captured, weighing under 100g, were marked using a modified version of the method originally developed by Cagle (1939). The marginal scutes of the carapace were assigned a letter (Figure B.1). Turtles were marked by using a small saw blade to cut a unique combination of notches in the marginal scutes of the turtle's carapace. This method of marking turtles is thought to be permanent in mature individuals, whereas the marks on juveniles gradually fade away as the turtle grows.

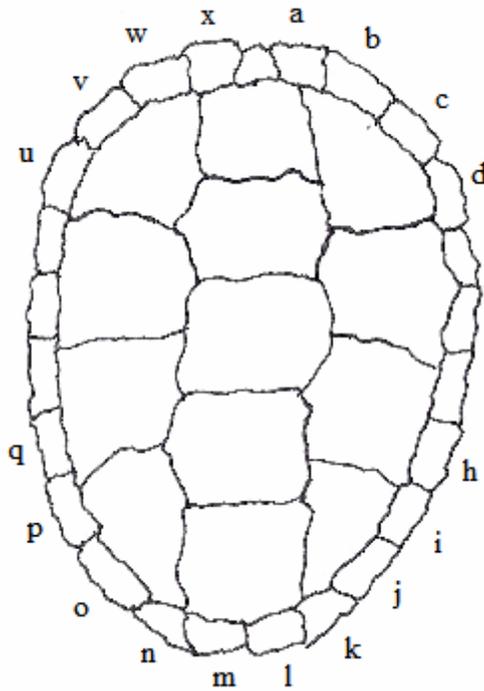


Figure B.1. An illustration of a turtle's carapace, displaying how letters were assigned to the marginal scutes.

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Appendix C

Bait Test

In studies of freshwater turtles, aquatic traps are often the primary method used to capture turtles and in order to successfully do this, traps must be baited with an attractant. The most popular bait used to trap turtles is sardines and has been cited in the methods of numerous publications (e.g., Parmenter, 1980; Smith and Iverson, 2002). However, others have had great success using can cat food as bait, which cost roughly a third of the price of sardines (P. King, personal communication). Therefore, a small study was conducted to determine which bait was the most effective for capturing turtles in aquatic traps.

A matched pairs design was used to compare baits during June of 2006 in Dargans' Pond. Two hoop net traps, one baited with sardines and the other baited with cat food, were placed approximately 2 meters apart at 4 trap sites, for a 24 hr period. Turtles were trapped at each site for 4 consecutive days for a total of 16 trap nights. Both, sardines and cat food produced 21 turtle captures, made up of two species (Table C.1). A chi-squared contingency table analysis showed that the type of bait has a significant effect on the turtle species captured ($\chi^2 = 5.44$).

Table C.1. The resulting turtle captures comparing the use of sardines and can cat food as bait in aquatic traps.

Species	Captures	
	Sardines	Cat Food
<i>Trachemys scripta</i>	11	18
<i>Sternotherus odoratus</i>	10	3
Total	21	21

Based on these results, both types of baits are equally effective in capturing turtles. Although, the type of bait used appears to have a significant effect on the species captured. This implies that the type of bait used in turtle traps can be selected based upon the target species being studied. If one was interested in studying *T. scripta*, then can cat food would be the most productive bait to use, whereas *S. odoratus* was more abundant in traps baited with sardines. However, when studying the species composition of turtle communities, a trapping bias may be introduced into the study when one bait is selected over the other. Therefore, future research should take a more comprehensive approach to identify the effects that the type of bait has on the species captured in aquatic turtle traps.

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