T-35 Final Report

NOTE: The final report for T-35 entitled "Identification of Diamondback Terrapin Habitats in South Carolina" was fulfilled by this thesis.

DIAMONDBACK TERRAPINS (*Malaclemys terrapin*) OF CHARLESTON, SOUTH CAROLINA: POPULATION ESTIMATE, SEX RATIOS AND DISTRIBUTION

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

MASTER OF SCIENCE

in

MARINE BIOLOGY

by

ELIZABETH BROYLES
AUGUST 2010

at

THE GRADUATE SCHOOL OF THE COLLEGE OF CHARLESTON

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ABSTRACT

DIAMONDBACK TERRAPINS (*Malaclemys terrapin*) OF CHARLESTON, SOUTH CAROLINA: POPULATION ESTIMATE, SEX RATIOS AND DISTRIBUTION

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Very little is known about the current population number, sex ratio, and distribution of Diamondback terrapin populations in Charleston, South Carolina estuaries. Terrapins were caught in the Ashley River, and population estimates were calculated using mark and recapture techniques and analyzed using the MARK program. Population size was estimated to be 3060 with a 95 % confidence interval of 1964-4156. This gives around 179-378 terrapins per km^2 of marsh habitat. The sex ratio was 1.7:1 male biased ($p < 0.001$). Investigations into changes in land usage were used to reveal reasons for change in terrapin abundance in the watersheds of the Ashley River, the Wando River and the Charleston Harbor from 1995-2009. The number of terrapins caught at all Wando River sites combined significantly decreased during the study period ($r = 0.83$, $p < 0.001$). There has been approximately 12.9 km^2 (10% of 127.72 km^2) of land use change in the Wando River watershed from 1996-2006. Diamondback terrapin abundance, estimated via catch per unit effort, has remained constant for most of the Ashley River and Charleston Harbor areas. Land use change has been minimal ($\leq$ 2%) in both of these watersheds during the same time frame. The Wando River, on the other hand, had a significant decline in terrapin CPUE and also had a much greater amount (10%) of land use change. Land use can encroach on terrapin habitats, nesting sites and impact food and foraging areas. If the declining trend of the terrapin population in the Wando River continues, regulatory intervention may need to be considered. This information on population size, sex ratios, and distribution can be used as a baseline to track long term changes in terrapin populations.

Acknowledgments

I would like to thank all of the members of my thesis committee for their continued support of my research while at the College of Charleston. I am grateful for my advisor Dr. David Owens for his guidance and expertise, without which I would never have been able to work with terrapins during my time as a graduate student. He was always willing to sit and talk about ideas and anything turtle related. I am thankful that I was able to work with Bill Roumillat and Erin Levesque, who took me in as a member of their DNR team. With their experience and knowledge, we were able to catch and tag more terrapins than I ever imagined. They were extremely helpful with information, and their guidance was invaluable in the creation of this project. Allison Welch was always spot on with ideas and explanations on how I could best convey the results of my project to others. Each person was an integral member of my committee and provided numerous insights into data interpretation, framing of ideas and statistical methodologies. My thesis was greatly improved by the comments of all of my committee members. I am also extremely indebted to the SC DNR Inshore fisheries division. They collected all of the terrapins used in my study. Specifically, John Archambault, Robin Freeman, Chris McDonough, Jonathan Tucker, and Henry DaVega helped out immensely with catching the terrapins. Without them this project would not have happened at all. I really appreciated the help of Joy Gerhard and Brock Renkas who were in the same situation as me, working while trying to produce a great thesis project. Conversations, explanations, and songs gave me a refreshing distraction from the stresses of class and writing. I want to thank Jordan Felber for his help with my GIS maps. I want to thank Pete Meier for training me on the boat. I also give many thanks to my friends and classmates who came out on the boat with me to record data while I tagged terrapins. I want to thank Brett Mills who kept my spirits up and made me smile when I was down and helped me see the big picture. I also want to thank my family who have been incredibly supportive and encouraging during my time as a graduate student. And finally, I want to thank anyone who ever saw my pictures from the field and said to me “wow, that's cool.” That definitely gave me motivation to keep going and reminded me how amazing this work really is.

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Animal Use and Permits.---

This work was conducted under collection permits from the South Carolina Department of Natural Resources (SC DNR) and animal care and use protocol IACUC-2008-017 approved by the Institutional Animal Care and Use Committee (IACUC) of the College of Charleston.

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Chapter 1: Diamondback Terrapin Ecology and Background

INTRODUCTION

Ecology of Diamondback terrapins.---

The diamondback terrapin, *Malaclemys terrapin* (Schoepff, 1793), is a semi-aquatic turtle that is found in brackish waters ranging from Massachusetts to the Gulf coast of Texas. Terrapins reside primarily in marshy areas and have a relatively small home range. This is the only emydid turtle in South Carolina, and in the world, that spends its entire life in estuaries.

Diamondback terrapins are divided into seven subspecies based on their geographic range. The subspecies *M. terrapin centrata* (Latreille, 1802) is found from Cape Hatteras to the northern part of Florida (Ernst et al., 1994). Terrapins in South Carolina are active from March through November, overwintering from December through February. During this brumation (winter dormancy) period , terrapins are presumed to rest along the bottom of the water or to be buried in the mud along the high tide line (Yearicks et al., 1981), however it has been shown that terrapins in the southern region may come out of brumation during warmer days of winter (Ernst and Lovich, 2009).

Diamondback terrapins have large hind legs with webbed toes for fast and efficient swimming, and *M. terrapin centrata* have highly varied coloration on their skin (gray, brown, yellow or white) and carapace (black, brown, gray, orange, olive or tan) (Conant and Collins, 1998). Their skin can also have dark spots, stripes, or blotches

(Ernst and Lovich, 2009).

Terrapins exhibit strong sexual dimorphism, with the females growing significantly larger than the males when mature. Due to this difference in size, their foraging niches only partially overlap. The males and smaller, immature females eat small periwinkle snails (*Littoraria irrorata*) while larger females eat the larger periwinkles along with crustaceans, fish, insects, and mollusks (Conant and Collins 1998, Tucker et al., 1995). Periwinkles and small blue crabs (*Callinectes sapidus*), also common in salt marshes, are the main food supply for terrapins in South Carolina (Tucker et al., 1995).

Terrapins mate in the spring, and females lay clutches of five to twelve eggs during the months of May to August. Upon hatching, baby terrapins move toward the closest marsh or shrubs and burrow under the vegetation and debris. It is thought that the juveniles seek refuge under mats of vegetation such as *Spartina spp.* (Lovich et al., 1991). Juveniles that are under three years of age are rarely seen in tidal creeks (Gibbons et al., 2001). Little is known about the life of juvenile terrapins up to sexual maturity when they are more often seen again.

Terrapins exhibit higher mortality as juveniles than adults. A variety of predators have been implicated in digging up and preying upon eggs and newly hatched terrapins. Predators include mammals such as raccoons (*Procyon lotor*) (Burger, 1977; Seigel, 1980; Butler, 2002), dogs (*Canis lupus familiaris*), foxes (*Urocyon cinereoargenteus, Vulpes vulpes*) (Burger, 1977; Tucker et al., 2001), rats (*Rattus norvegicus*) (Draud et al., 2004), and armadillos (*Dasypus novemcinctu*) (Butler, 2002), birds such as laughing gulls (*Larus atricilla*) (Burger, 1977), fish crows (*Corvus ossifragus),* American crows *(C.*

branchyrhynchos), and boat-tailed grackles (*Quiscakus major*), and invertebrates such as red imported fire ants (*Solenopsis invicta*) (Butler et al., 2004) and ghost crabs (*Ocypode quadrata*) (Arndt, 1991). Raccoons and foxes are also common predators of females that have left the water to nest (Seigel, 1980, Tucker et al., 2001). Adult terrapins are also preyed upon by aquatic predators such as dolphins, sea turtles, and crocodilians (Ernst and Lovich, 2009). Some animals may cause damage to terrapins such as sharks (adult *Sphyrna tiburo* and juveniles of other species), blue crabs (*C. sapidus*), stone crabs (*Menippe mercenaria*), and toadfish (*Opsanus tau*) (Cecala et al., 2008). Human harvesting still takes place in some areas. Chinese restaurants in New York City were conservatively estimated to sell 10,000 terrapins a year in 1988, collected mostly from Virginia, the Carolinas, and New Jersey (Gerber, 1988).

Terrapin Sex Ratio.---

Diamondback terrapins are sexually dimorphic with the adult females (carapace length 15-22.9 cm) being noticeably larger than the adult males (carapace length 10.0-14.0 cm) (Conant and Collins, 1998). The position of the cloaca in the mature females is anterior to the rear edge of the carapace, while in the mature males, the cloaca is posterior to this line (Tucker et al., 2001); this allows for easy sexing of the adult terrapins. Confusion is possible when looking at immature terrapins since small females appear very similar to males. Secondary sexual characteristics can help determine sex in this case; males have thicker and longer tails than females, while females have a wider head than males (Seigel, 1984).

Sex ratios in *M. terrapin* have been documented as being biased toward one sex, or the other. Male bias seems to be typical in Carolina terrapin studies (Gibbons et al., 2001; Butler, 2002). Lee (2003) also found a 1.7:1 male-biased sex ratio in the Charleston area. Levesque (2000) found a 3.14:1 male-biased sex ratio in the Wando River, which is adjacent to the Ashley River in Charleston, SC. However, female-biased sex ratios have been reported in other areas. There was a 2:1 female-biased sex ratio in Chesapeake Bay (Roosenburg et al., 1997) as well as in Long Island, NY (Morreale, 1992) and a 5:1 female biased (Seigel, 1984) in Merritt Bay, Florida.

Temperature dependent sex determination (TSD) has been established in diamondback terrapins (Roosenburg and Kelley, 1996; Hart and Lee, 2006) and it can affect the primary sex ratios. TSD is different from genetic sex determination (fixed upon conception) because the sex of the embryo is determined after the embryo is exposed to certain temperatures during incubation. TSD is found in other reptiles such as alligators, some turtles and the tuatara (Huey and Janzen, 2008). In many reptiles, the sex is determined during the middle third of the embryos development (Janzen, 1994). Temperatures in early July are influential for terrapin gender determination (Auger, 1989). Females result from warm temperatures while males are the result of cooler temperatures. Studies have shown that eggs incubated at 24-26°C produce all males, while eggs incubated at 30-32°C produce all females (Ewert and Nelson, 1991; Roosenburg and Kelley, 1996). This indicates that nest placement has a direct effect on the nest temperature and sex ratio of the hatching brood. Nests laid in shady areas will be cooler and produce mostly males, while nests laid in direct sunlight will produce mostly females (Hart and Lee, 2006).

Although TSD can affect primary sex ratio, skewed sex ratios may also be due to sampling bias, differential mortality, or differential maturity of the sexes (Lovich and

Gibbons, 1990). Crab pots are thought to cause a decline in the males because the smaller males enter crab pots and drown (Grosse et al., 2009; Roosenburg, 2004; Roosenburg et al., 1997) whereas road mortality is thought to cause a decline in adult females that cross roads in order to find adequate nesting areas. Males also mature more quickly (3-4 years) than females (5-7 years) (Lovich and Gibbons, 1990), which could impact the actual effective adult sex ratio.

Anthropogenic Effects.---

Anthropogenic interactions have been generally detrimental for *M. terrapin* populations over their common history. Terrapins were used in turtle soup, and cooked in other ways, for much of the late 1800s and early 1900s. Native Americans also ate terrapins regularly since terrapin bones are found in middens. The species nearly went extinct in the early 1920s due to over-harvesting (Carr, 1952). Major causes of contemporary terrapin population declines include road mortality (Szerlag and McRobert, 2006), drowning in crab pots (Siegel and Gibbons, 1995; Hoyle and Gibbons, 2000), drowning in eel pots (Radzio and Roosenburg, 2005), nesting habitat degradation and loss (Roosenburg et al., 1997) and legal and illegal harvest (Gerber, 1988). A population decline has been documented in a well-studied area of the lower Kiawah River in South Carolina where extensive development over the past thirty years may be the problem (Gibbons et al., 2001). Another study done at the Kiawah River site has shown that the terrapin population has been adversely affected by recreational crab pots (Hoyle and Gibbons, 2000). Since terrapins are reptiles, they must have access to air to breathe, while blue crabs use their gills to obtain oxygen from the water, so they do not need access to air. For this reason most crab pots are made to sit entirely below the surface of the water

(Roosenburg et al., 1997). Terrapin mortality in crab pots is caused by the terrapins being attracted to bait or the trapped crabs, or possibly to other terrapins already in the trap. Regardless, they enter the trap and subsequently drown. Abandoned or lost crab pots, also called “ghost” crab pots, often remain intact in the estuarine environments where they can continually catch and kill numerous terrapins and other organisms (Guillory and Prejean, 1998). Adult females are usually too large to enter the crab pots, so consequently, preferential selection of smaller males and juveniles in crab pots can cause the population to become more skewed towards females (Hoyle and Gibbons, 2000).

South Carolina Department of Natural Resources 2009-2010 Freshwater and Saltwater Rules and Regulations (www.dnr.sc.gov/fish) states that the commercial sale of terrapins is prohibited. In 2003, the diamondback terrapin was listed as a species of lower risk/near threatened by the International Union for Conservation of Nature and Natural Resources (IUCN). Terrapins are also currently listed in the South Carolina Comprehensive Wildlife Conservation Strategy (CWCS) as a species of concern and are considered a high priority species. “Species of Concern” is a term used as a conservation tool to direct research toward listed animals that are not protected by law.

Terrapins in Charleston, SC.---

The population of *M. terrapin centrata* in the Charleston, SC area has not been well described. Very little is known about their distribution in the coastal areas or current population status, including relative numbers, sex ratios, and maturity stages. Consequently, there is a need to survey this population to determine its population size, sex ratio, and distribution. There is also a need to determine if and how land use change (land and habitat degradation, increased impervious surfaces, etc.) affects terrapin populations.

The aim of this study was to estimate population size, sex ratio, and the distribution pattern of *M. terrapin centrata* in the Ashley River area of Charleston, SC. In a two-year study, capture-mark-recapture techniques using PIT tags were used to estimate their populations as well as to develop a new method of long term tagging and population monitoring for diamondback terrapins. In addition, terrapin capture data from 1996-2009, along with development maps of the water sheds of the Ashley River, the Wando River and the Charleston Harbor from 1996 to 2006, were examined for changes in terrapin abundance and changes in land use. The information on population size, sex ratios, and distribution can be used as a baseline to track long term changes in terrapin populations. Data on changes in land use and terrapin abundance could be used to determine if regulations regarding habitat degradation, as well as possible additional protection, are needed for this species.

Chapter 2: Population Estimate and Sex Ratio for the Ashley River in Charleston, SC

INTRODUCTION

Terrapin population numbers have been a concern since the early 1900s. Terrapins were used in turtle soup for much of the late 1800s and early 1900s and the species nearly went extinct in the early 1920s due to over-harvesting (Carr, 1952). They are presently considered a 'species of concern' in nine states (LA, MS, AL, GA, NC, DE, NJ, CT) including South Carolina, and are threatened or endangered in two states (MA, RI).

Population studies are needed to identify any changing trends in terrapin numbers. Estimates were 2,778-3,730 in the Patuxent River Estuary of the Chesapeake Bay area (Roosenburg et al., 1997), 3,375 in Mississippi (Mann 1995) and 1,655 in Canary Creek in Sussex county Delaware (Hurd et al., 1979), 212 and 404 for two sites in Merritt Island, Florida (Seigel, 1984), and 344 and 341 at two sites in New York (Morreale, 1992). The sizes of these areas vary so they cannot be compared with each other. An initial estimate is needed for the Charleston, SC area as a baseline number that can be compared to future studies.

Identification of individual terrapins is important when trying to estimate population parameters; counting the same terrapin twice can cause inaccurate estimates. Tagging or marking animals can be useful for identification of individuals. Passive integrated transponder (PIT) tags are small plastic or glass encased microchips that are injected into the terrapin subcutaneously and can be read with a hand-held reader. Each tag has a unique number that is used for identification of the terrapin tagged. PIT tags have been utilized successfully in recent terrapin mark-recapture studies (Szerlag-Egger and McRobert, 2007; Hart and McIvor, 2008). PIT tagging methods have also been used previously with other aquatic animals. For example, PIT tags were successfully used to mark salmon (*Oncorhynchus nerka* and *O. tshawytscha*) (Prentice and Park, 1984). These authors concluded that the tag did not affect survival or growth of the fish. PIT tags were also used in a study to determine the likelihood of tag loss in leatherback sea turtles (*Dermochelys coriacea).* These tags were used as the permanent tag for identification of the turtle, and were especially useful if the temporary flipper tags were lost (Rivalan et al., 2005).

There have been several tagging methods used to identify terrapins. The notching of marginal scutes is the most widely used method for identification of turtles (Levesque, 2000; Gibbons et al., 2001; Dorcas et al., 2007; Hart and McIvor, 2008; Roosenburg and Allman, 2003), but over time the notches can become worn and hard to read (Roosenburg and Allman, 2003). Coding mistakes by researchers are also possible (D. Owens, pers. comm.). The terrapin's shell may also become nicked, making the identification difficult (Roosenburg and Allman, 2003). Molecular tags have been proposed such as taking blood samples for microsatellite analysis (Hart and McIvor, 2008), but this is costly and requires an additional time in the field taking blood and in the lab processing it. Other tagging procedures include binary coded wire tags (Roosenburg and Allman, 2003) but examination of these tags can only be done by removing the tag from the animal or with a portable x-ray unit. Having considered all of these options, PIT tagging was determined to be the most reliable and affordable way to positively identify the terrapins in our specific locations.

Terrapin sex ratios have varied from male to female biased depending on the study (Seigel, 1984; Morreale, 1992; Roosenburg et al., 1997; Levesque, 2000; Gibbons et al., 2001; Butler, 2002; Lee, 2003). With terrapins exhibiting temperature dependent sex determination and with continuing threats of global climate change, a shift in sex ratios toward more female hatchlings may be occurring. Anthropogenic mortality can have a gender specific effect. Crabs pots kill mostly male terrapins (Hoyle and Gibbons, 2000), while road mortality affects females (Szerlag and McRobert, 2006). If sex ratios shift in one direction, it may be a clue as to what is happening to the population.

The objectives of this study were to estimate (i) the population size and (ii) the sex ratio of terrapins in the Ashley River in Charleston, SC. These objectives were addressed by using PIT tags in a capture, mark, recapture experiment. The sex of each terrapin caught was recorded and used to determine the ratio of males to females in the Ashley River population. This information will be used in order to establish baseline numbers for future comparisons and studies. An additional objective was to determine the retention rate of PIT tags. This objective was addressed using two control experiments involving captive terrapins.

MATERIALS AND METHODS

Capture, mark, recapture study.---

The terrapin capture methods were implemented in collaboration with the SC-DNR Inshore Fisheries section. Their standard sampling procedure, used to catch estuarine fishes, has proven to be a good capture method for terrapins, a common by-catch species (Levesque, 2000). The sampling protocol is briefly described below. Each month a sampling set was randomly chosen from a larger group of predetermined sites along the periphery of a 9.3 km stretch of the Ashley River in Charleston, SC (Table 1; Fig. 2). All sites were available to be selected each month. Terrapins were caught and tagged during one designated day during falling tide, and then recapture efforts and additional tagging occurred on approximately the same day the following month (Table 2). Capture sites were recorded by the numerical system established by the DNR Inshore Fisheries group.

Two Florida net boats, with an outboard engine in a center console at the bow, were used to rapidly set separate trammel nets along the banks of the salt marsh. Each boat set seven different sites per sampling day. Trammel nets (182m long and 2m deep) consisted of three layers of netting, a smaller inner panel of webbing (64 x 64 mm) in between two outer layers of larger webbing (178 x 178 mm). The webbing was attached to a float line on top and a lead line on the bottom in order to keep the net upright in the water column. Single anchors attached to each end of the net were dropped at the edge of the marsh, and the net was set in a gentle curve to enclose a section of the shallow habitat. The nets were placed in water $\leq$ 2 m deep. Once the net was set, the boat traveled between the net and the bank, and the water was disturbed at the surface using long, wooden poles as well as banging the hull of the boat. This intensive vibration of the water behind the net appeared to cause animals to retreat into deeper water, thereby getting caught in the net. Nets were then immediately retrieved. Ideally each boat attempted to set seven different sites for a total of 14 sites each sampling day. Weather, anglers near the sampling location, and low water levels sometimes prohibited certain selected sites from being set on particular days, in which case they were replaced with predetermined alternate sites.

Captured terrapins were placed in 22 liter buckets for temporary holding for no more than one hour. For each terrapin captured, straight line carapace length (CL), carapace width (CW), plastron length (PL), and head width (HW) were measured with calipers. Each terrapin was weighed using a spring scale. The sex of the terrapin was determined by overall size of the animal (females > 14 cm) and by the location of the cloaca relative to the carapace, along with secondary sexual characteristics. Female terrapins have the cloacal opening anterior to the rear edge of the carapace, and males have the cloacal opening posterior to the rear edge of the carapace. Males have a thicker, longer tail than females, and females have a wider head than males (Seigel, 1984).

Each terrapin was implanted with a plastic 11 mm PIT microchip tag (Ensid polymer 11mm FDX-B PIT tags). The PIT tag was injected sub-dermally into the right rear leg of the animal using an 11mm single shot implanter (Hallprint). Prior to injection, the site was scrubbed with isopropyl alcohol to reduce the likelihood of infection. Each terrapin was digitally photographed and the identification number of the PIT tag was recorded. The animal was then immediately released at the site of capture.

Terrapins caught during the recapture effort were scanned for PIT tag identification. Recaptured animals were also checked for tagging scars and infections at the tag site. Those determined to be recaptures were re-measured while non-tagged terrapins were measured and tagged using the procedures described above. Estuary physical parameters were measured for each capture location. Water quality was characterized at each station by measuring salinity (refractometer), air temperature (stem thermometer), dissolved oxygen and water temperature (YSI, 550A Dissolved Oxygen Instrument, YSI Incorporated, Yellow Springs, OH).

A goal of approximately 500 PIT tagged terrapins each season (2008 and 2009) was established to provide an adequate sample size for statistical analysis. Tagging efforts took place during the months of May 2008 - September 2008 (sampling season one) and April 2009 - September 2009 (sampling season two). Recapture efforts for terrapins tagged during sampling season one took place from May 2008 - December 2009. Recapture efforts for terrapins tagged in sampling season two took place from May 2009 - December 2009.

PIT tags used in sampling season two (2009) were recalled due to a manufacturer's error in which the tags may or may not have been readable (see Appendix 1). Of the 343 terrapins tagged in the 2009 season there were only 2 recaptures, which is inconsistent with the recaptures from the 2008 season. The low number of recaptures would clearly skew the population data. Given the manufacturer's recall, it appears that the low number of recaptures may have been due to a large number of unreadable tags. Because faulty tags violate the assumptions of the Jolly-Seber model (properly identifiable tags), the entire 2009 tagged terrapin data set was not included in estimates of population size.

Statistical Analysis.---

Mark and recapture data were statistically analyzed using the program MARK (White, 2006). The POPAN (Population analysis) module was used to determine population estimates using the Jolly-Seber (JS) method (Pollock et al., 1990; Lebreton et al., 1992). The JS model assumes that the captured and marked subject animals have the same probability of being caught as the unmarked animals. Other assumptions for this model include: properly identifiable tags, retention of tags (no tag loss), equal survival rates among tagged and non-tagged animals between sampling occasions, and a stable study area. These assumptions allow for an estimation of population size (N_i).

Data required for estimate:

n_i - Number of animals captured in sample i, both marked and unmarked, $n_i = m_i + u_i$

u_i - Number of unmarked animals captured in sample i

m_i - Number of marked animals captured in sample i

s_i = number of animals released at sample i

R_i = number of the s_i individuals released at sample i and caught again in a later sample

z_i = number of individuals marked before sample i, not caught in sample i, but caught in some sample after i

The survival rates of both marked and unmarked animals are represented by the φ_i values. These are calculated between successive occasions. The p_i values represent the probability of capture of marked and unmarked animals at the ith occasion. M_i and U_i are unknown parameters that represent the marked and unmarked individuals in the population, respectively, alive at the time of occasion i. (Cooch and White, 2006).

M_i can be estimated as: $$M_i = m_i + \frac{(s_i + 1) z_i}{R_i + 1}$$

And finally, the population size before time i (N_i) can be estimated by:

$$N_i = \frac{M_i (n_i + 1)}{m_i + 1}$$

(Seber, 1982)

This model takes into account the intervals between collection dates as described above. Variations of constant and time dependent p_i and φ_i values were tested and the model with the best AIC value was used for all estimates.

Sex Ratio.---

The sex of the terrapins caught during both sampling seasons was used to analyze sex ratio. Chi-square goodness of fit analysis tested for deviation from a 1:1 sex ratio.

Control experiments.--

A control experiment was used to determine the retention rate of PIT tags in this study. A control group of 20 terrapins (10 males, 10 females) were caught on October 8, 2008 in Grice Cove of Charleston Harbor, SC (Fig. 1) using trammel nets. Captured terrapins were measured, weighed and tagged, following the procedures described below. Terrapins were held outdoors at the Grice Marine Laboratory, Charleston, SC in four 1200 liter (300 gallon) Rubbermaid tanks, with five terrapins per tank. One tank contained only males, another contained only females, and the final two tanks contained mixed sexes. Each tank contained approximately 400 liters (100 gallons) of water with a salinity of approximately 18-21 ppt. The water was brought in from the Charleston Harbor, settled out in a large tank and filtered through a 1 µm canister filter and then continuously run through a UV sterilizer. Each week, approximately ten percent of the water was removed and replaced. Tanks were covered with a heavy wire mesh (5.1 cm x 5.1 cm) to exclude predators and to prevent escape. Turtles were examined and PIT tags checked every other day for 49 days. Terrapins were fed Mazuri© Freshwater Turtle Diet gel food, which includes a vitamin supplement, and small pieces of local fish three times per week for the duration of the experiment. Terrapins were given *ad libitum* access to the food. Excess food was removed when the water was changed. At the end of the experiment (November 26, 2008), the terrapins were reweighed and inspected for scarring and infection at the injection site, then released back to Grice Cove.

An additional eight terrapins were tagged in May 2008 and held at the South Carolina Aquarium in order to determine PIT tag migration within the terrapin's body. These terrapins were tagged in a clean laboratory and were injected with antibiotics in order to reduce infection possibilities. The initial wound from the injection was closed using epoxy glue to encourage healing and to prevent bacterial infection from moving between the terrapin and the environment. The tagged terrapins were held in the marsh exhibit and cared for by aquarium staff. These terrapins were scanned for PIT tags throughout the duration of the experiment (May 2008-Dec 2009).

RESULTS

Population Estimate.---

During sampling season one, 428 terrapins in the Ashley River were PIT tagged. From this group of tagged terrapins, there were a total of 27 recaptures in 2008 and 26 recaptures in 2009. During sampling season two, a total of 343 terrapins were tagged, of which two were recaptured. Due to the likelihood that some of the PIT tags used in the 2009 sampling season were faulty (see methods), the 2009 tagging data were not used in the analysis of the gross population estimate using Program MARK. Only recaptures from the terrapins tagged in sampling season one (2008) were used.

Based on recapture data from 2008, the gross population estimate was 4864 with a 95% confidence interval of 3017- 6711. Including both 2008 and 2009 recaptures of terrapins tagged in season one, the gross population estimate was 3060 with a 95% confidence interval of 1964 – 4156. These estimates assume zero tag loss.

Terrapin movement was minimal in the Ashley River. Of the 55 recaptures, 42 were in the same site as their initial capture (Table 3). Of the fifteen that were caught at the site other than the initial capture site, ten were female and five were male. Three recaptured female terrapins traveled 1.5 km over one month (July- Aug), 2.1 km over 3 months (Aug- Sept), and 3.5 km over 1 year (May 08 – May 09), respectively. These were the longest distances traveled by terrapins recaptured in this study.

Sex Ratio.---

During sampling season one, 254 male terrapins and 174 female terrapins were captured giving the population a 1.5:1 sex ratio. This sex-ratio was significantly male biased ($p = 0.0001$). There was no significant difference in the sex ratio of terrapins recaptured in 2008 (14 males and 13 females) or 2009 (14 males and 12 females). In sampling season two, 228 males and 115 females were captured giving the population a 1.98:1 sex ratio, which was also significantly male biased ($p < 0.0001$). The two recaptured terrapins from this tagging season were female. Combining both sampling seasons, there were a total of 482 males and 289 females tagged giving a significantly male biased sex ratio (1.7:1; $p < 0.0001$).

The sex ratio varied month to month during the study. Five months were significantly male biased, while the other months did not differ significantly from a 1:1 sex ratio (Fig. 3).

Control experiments.---

All 20 terrapins kept at the Grice Marine Laboratory retained their tags for the 49 day control study (a 0% loss rate). One out of the eight terrapins kept at the South Carolina Aquarium lost its tag during the first year (a 12% loss rate). Loss rates from the terrapins at the two locations were not combined due to the difference in duration and tagging protocol of these experiments. When the more conservative 12 % loss rate is applied to the sampling season one data, the gross population estimate for 2008 was 4315 with a 95% confidence interval of 2781- 5848. The gross population estimate for 2008 and 2009 combined with a 12 % loss rate (not including the second year's tags) was 2691 with a 95% confidence interval 1982 – 3399.

DISCUSSION

Population Estimate.---

The number of terrapins in the sampled 9.3 km section of the Ashley River with 11 km^2 of adjacent marsh land was estimated based on one year of PIT tag marking (2008) and two years of terrapin recaptures (2008-2009). Population size was most conservatively estimated to be 3060 with a 95 % confidence interval of 1964-4156. This gives approximately 211-447 terrapins per km of river and 179-378 terrapins per km^2 of marsh habitat. Other terrapin studies based on mark-recapture methods have yielded similar population estimates: Using modified crab pots, hand captures, and cast nets, Butler, (2002) estimated 3147 terrapins in northeastern Florida. In 1991, 1717-2895 terrapins were estimated in a 10 km stretch of the Patuxent River Estuary of the Chesapeake Bay (Roosenburg, 1991), which gives an estimate of 172-290 terrapins per km of river. In 1997, 2778-3730 terrapins were estimated in the same area (Roosenburg et al., 1997). This gives an estimate of 278-373 terrapins per km of river. These numbers are not different from the numbers in our current study although terrapins in Roosenburg's study were caught using a variety of methods such as bank traps, fyke nets, gill nets, standard and modified crab pots, and by hand (Roosenburg et al., 1997). The Chesapeake Bay and northeastern Florida terrapin estimates most closely resemble the Ashley River estimates of 2009.

In 1979, an estimate of 1655 was found for a 0.9 km of creek, which gives 1800 terrapins per km in Canary Creek in Sussex county Delaware (Hurd et al., 1979). These results are different from our study. The higher number in Delaware could be attributed to the sampling method of trawling the 0.9 km of river up to four times during low slack tide to increase the capture number. Estimates of 212 and 404 for two sites in Merritt Island, Florida (Seigel, 1984), and 344 and 341 at two sites in New York (Morreale, 1992) were also noted but the areas of study were not defined so comparisons can not be made.

The number of terrapins in the Ashley River may be indicative of an optimal terrapin habitat. The area around the Ashley River has had minimal recent urban sprawl compared to the surrounding water sheds (see Chapter 3). The extensive marsh land (11 km^2) surrounding the Ashley River area where the terrapins were captured (Fig. 4) may also provide excellent access to locations for nesting and foraging.

Terrapin movement was minimal in the Ashley River. Approximately seventy-five percent of the 55 recaptures were caught in the same site as the initial capture. Site fidelity has been previously reported to be as high as 97% among 205 marked terrapins in Kiawah, South Carolina (Gibbons et al., 2001) and 40% among 547 marked terrapins in Sussex County, Delaware (Hurd et al., 1979), and Estep (2005) noted high site fidelity in her radio and sonic tracking studies of terrapins in the Charleston Harbor. Thus, our study reaffirms the pattern of very high site fidelity of these animals. High site fidelity could be due to abundant food, available mating partners, general habitat quality or a form of habitat imprinting such as is seen in sea turtles and anadromous fishes (Papi, 1992).

Sex Ratio.---

The sex ratio found in the Ashley River in Charleston River was 1.7 males to every female. Male bias seems to be typical in Carolina terrapin studies (Gibbons et al., 2001; Butler, 2002). Lee (2003) also found a 1.7:1 male-biased sex ratio in the Charleston area. Levesque (2000) found a 3.14:1 male-biased sex ratio in the Wando River, which is adjacent to the Ashley River in Charleston, SC. However, female-biased sex ratios have been reported in other areas. There was a 2:1 female-biased sex ratio in the Chesapeake Bay area (Roosenburg et al., 1997) and in the Long Island, NY area (Morreale, 1992) and a 5:1 female bias (Seigel, 1984) in Merritt Bay, Florida.

The relatively high number of male terrapins may indicate that crab pot mortality is not a large issue in the Ashley River, although there were high densities of crabs pots located there. These crab pots were located in deeper waters where males don't normally go (personal observation). Alternatively, the lower number of female terrapins could be due to greater risks of mortality during nesting periods such as road mortality (Szerlag and McRobert, 2006), terrestrial predators (Burger, 1977; Seigel, 1980; Feinberg and Burke, 2003; Butler, 2002; Draud et al., 2004) and boat strikes (Gibbons et al., 2001), or it may be due to differential maturity rates (Lovich and Gibbons, 1990) or undocumented impacts of temperature dependent sex determination.

Lovich and Gibbons (1990) found a 1.78:1 male-biased sex ratio in Kiawah, South Carolina. They determined that differential age at maturity was a "satisfactory explanation" for a male bias in terrapin populations. Studies show that males mature around age three at 90 mm plastron length and females mature around age six at 132-176 mm plastron length (Cagle, 1952; Lovich and Gibbons, 1990). Assuming similar life spans, the fast maturation rate of males would lead to more sexually active males in the population and therefore possibly more males captured. Lovich and Gibbons (1990) ruled out biased sampling, skewed primary sex ratios (although TSD had not been well established in terrapins at the time), differential mortality rates, and differential immigration and emigration. Gibbons et al. (2001) reported that the Kiawah populations were still male-biased despite increased crab pot usage. However, Dorcas et al. (2007) reported an increase in the percent of female turtles captured by year in his study, from approximately 45% in the mid-1990s to more than 80% in 1997. This apparent decrease in male terrapins may have been related to increased crab pot usage in the area (Dorcas et al., 2007) or global climate change, which could cause increased incubation temperatures (establishing more females). Cagle (1952) showed a similar male bias in a Louisiana population of terrapins, but suggested that this skew may have been due to females leaving to find nesting areas.

Temperature dependent sex determination (TSD) in terrapins has been noted in several studies (Roosenburg and Kelly, 1996; Hart and Lee, 2006). Cooler temperatures in the Kiawah Island, SC and Charleston, SC nesting areas could cause a higher number of males in these populations. Nest placement can also have a direct effect on the nest temperature and possible predation risks. Terrapin nests laid in areas of vegetation cover are more likely to produce males, due to the cooling effects of shade, while nests laid in direct sunlight are more likely to produce females. Vegetation close by could be helpful to hatchlings (and un-hatched eggs, for that matter) by providing immediate coverage from predators. This could lead to a male biased sex ratio in terrapins in this study.

Another possible explanation for the male-biased sex ratio is female movement. Movement due to nesting or mating may cause females to be inaccessible to our nets. Tucker et al. (2001) found that females were more likely than males to move along tidal creeks, which we did not sample, and that males showed higher site fidelity regardless of creek position. A possible difference in foraging behavior may also influence the availability of female terrapins, since they tend to spend more time in deeper water (Roosenburg et al., 1999). Estep (2005) mentions five female terrapins that were sporadic residents of Grice Cove, Charleston, SC. These terrapins were detected using radio transmitters during late April, then mid June, and again in early September. There were spans of 3- 8 weeks between each detection when these terrapins were absent from the Cove. She reasoned that these terrapins lived outside of the cove but returned for mating and nesting. The three terrapins that traveled the longest distance in my study were all females. The movement and behavior of female terrapins could also cause them to be harder to catch in the trammel nets.

The significant difference in male and female terrapins caught in the months of June 2008 and March, April, May, and Nov 2009 (Fig. 3) is likely due to mating aggregations. Mating occurs in the spring and early summer and it has also been noted in the fall before hibernation (D. Owens pers. comm.). Other turtles, including the musk turtle (*Sternotherus odoratus*), have a second peak in mating in the fall (Mendonça, 1987). Gibbons et al. (2001) also found that male terrapin activity peaks in April and again in October. Mating aggregations and sex specific movement patterns may be the reasons that there were increased males during these times. This may also explain why the overall sex ratio was male biased.

The nearly equal number of male and female recaptures is much harder to explain. Why would recaptured terrapins show a different sex ratio than those initially caught? One possibility is that the sample size for recaptures was too small. A recapture number closer to the total capture number would give a more accurate sex ratio for the population (Allan Strand, pers. comm.). Alternatively, perhaps males learn to avoid the nets. It has been observed that terrapins can avoid the nets in some circumstances (Dave Owens pers. comm.). The PIT tagging could have caused more male mortality since the males are smaller than the larger, robust females, but this is unlikely as the control experiments indicated no loss of life or limbs to males or females due to tagging. The males may be foraging more widely or using a different part of the river system at different times during the year. Males may be residing in the smaller creeks and moving into the river during months when females are more available to mate. They may not move out of the upper creeks as often as the more mobile females. This could explain why there are so many new and recaptured male terrapins during the mating months. In this situation, the recaptured male and female terrapins are more likely to be the year long residents of the river. Lovich and Gibbons (1990) also reported an equal probability of recapturing a male or a female in an apparently male-biased population, but did not discuss why the probability of recapturing a male or a female was the same. The highest numbers of recaptures were caught during the months of May (18 recaptures) and July (9 recaptures).

Control Experiment.---

In the Grice lab control experiment there was no tag loss among the 20 terrapins held in the outdoor tanks, however there was one tag lost among eight terrapins kept at the SC Aquarium over a much longer period. These terrapins were moved around more than normal due to a leak in the marsh exhibit where they were kept during this experiment. This excess handling and small confinement may have caused undue stress and activity that could have resulted in the single PIT tag loss. Studies of PIT tags in wild fish have found that if tag loss did occur, it was immediate and the result of the expertise of the tagger (Clugston, 1996; Buzby and Deegan, 1999). In the current study, the tag did not come out of the terrapin's leg until ten months after the initial tagging. The wound from the tagging event was completely healed. Also, all of the terrapins tagged in the aquarium were tagged by the same individual. The possible loss of PIT tags in the actual field study will never be known, since the PIT tags were the only identification tag marking the terrapins. In future studies, the combined use of PIT tagging and an external mark (e.g., notching marginal scutes for a cohort or individual mark) can be used to assess tag loss in the field.

CONCLUSION

This study showed a population estimate range of 1964-4156 terrapins for an 11 km^2 area of marsh along a 9.3 km stretch of the Ashley River with a 1.7:1 male bias. This gives approximately 179-378 terrapins per km^2 of marsh habitat. There was minimal movement between capture locations with 75% site fidelity over the two season study.

The male bias seen in this study was similar to other South Carolina terrapin studies (Gibbons et al., 2001; Butler, 2002). Bias may be due to differential maturity rates, TSD or habitat partitioning between the sexes. Although this does not match with Fisher's (1930) 1:1 optimal sex ratio that indicates that there is a stable population with each sex having equal reproductive fitness, many reptiles have shown successful sex biased populations (Ferguson and Joanen, 1983; Shine and Bull, 1977). It is important to realize that many reptiles with TSD have hardly changed appearance in 200 million years (Deeming and Ferguson, 1989). Terrapin fossils have been found in SC from the Pleistocene epoch (2.588 million to 12000 years ago) (Dobie and Jackson, 1979). They appear to be well adapted for survival even without a 1:1 sex ratio. Even so, future monitoring of the sex ratios of the terrapins in the Ashley River, and other locations, is paramount to detecting changes that could affect the future of the population. Skewed sex ratios offer a clue to impacts on the terrapin population of an area; be it crab pots, road mortality or some other sex dependent threat. Consequently, sex ratio changes can also be used to determine what regulations ought to be implemented to help protect this species.

Chapter 3: Distribution of Terrapins in Charleston, SC Estuaries

INTRODUCTION

Anthropogenic interactions have been generally detrimental for *M. terrapin* populations over their common history. Terrapins were used in turtle soup, and cooked in other ways, for much of the late 1800s and early 1900s and prior to that by native cultures as well. The species nearly went extinct in the early 1920s due to over-harvesting (Carr, 1952). Major causes of contemporary terrapin population declines include road mortality (Szerlag and McRobert, 2006), drowning in crab pots (Siegel and Gibbons, 1995; Hoyle and Gibbons, 2000), drowning in eel pots (Radzio and Roosenburg, 2005), nesting habitat degradation and loss (Roosenburg et al., 1997) and legal and illegal harvest (Gerber, 1988).

Changes in land use associated with development may be a particular problem for terrapins. More commercial buildings, houses and paved surfaces can cause runoff of excessive fresh water or man-made materials such as hydrocarbons from vehicles, pollutants from lawn culture, and sediments from construction (Templeton et al., 2010). Development and pollution can attract predators such as raccoons and dogs, destroy feeding grounds and limit nesting ground availability (Ernst and Lovich, 2009). There may also be an increase in human water-related activities such as boating and fishing, which could impact local terrapin abundances. For example, boating can result in outboard motor strikes, which are sometimes fatal to terrapins (Ernst and Lovich, 2009).

A population decline has been documented in a well studied area of the lower Kiawah River in South Carolina where extensive human community development over the past thirty years may be the primary problem (Gibbons et al., 2001).

Salinity and water temperatures vary greatly within and among the range of terrapins. Butler (2002) found terrapins in the northeastern Florida area in salinities ranging from 4-35 ppt and water temperatures ranging from 12-33 °C. High salinity and low water temperatures can affect a terrapins' appetite and behavior (Davenport and Ward, 1993). Salinity and water temperature can change over time. These changes can be attributed to weather patterns (Schmidt and Luther, 2002; Wilkinson, 1996) or increased runoff (Nuttle et al., 2000). Differences in salinity and water temperature over time could be a cause of long term terrapin abundance changes.

Terrapin distribution in the Charleston, SC area has not been well described. Because this is a developed coastal area, terrapins may be affected by ongoing changes in land use. Investigations into changes in land usage were used in an attempt to reveal possible reasons for change in terrapin abundance and distribution in the watersheds of the Ashley River, the Wando River and the Charleston Harbor. The objectives of this study are (i) to assess changes in terrapin abundance, salinity, and water temperature in these water systems from 1995 to 2009 and (ii) to determine the amount of development along the Ashley River, the Wando River and the Charleston Harbor from 1996 to 2006. Development maps of these water systems were also evaluated for changes in land use in order to compare these changes with changes in terrapin abundance, as indicated by terrapin captures by the South Carolina Department of Natural Resources (SC DNR). These studies and observations may be useful for management and regulation purposes or for future research.

METHODS

Distribution.---

The terrapin capture methods were implemented in collaboration with the SC-DNR Inshore Fisheries section. Their standard trammel net sampling procedure, used to catch estuarine fishes, has proven to be a good capture method for terrapins, a common by-catch species (Levesque, 2000). Methods for terrapin capture were the same as discussed in chapter two.

From 1995 to 2009, the SC DNR Estuarine Finfish section has recorded the number of terrapins captured as bycatch while performing routine monthly sampling of estuarine fish. Sampling was done by trammel net, as described previously (Chapter 2; see also Levesque, 2000). Terrapins were captured during falling tides at sites selected using stratified random sampling, with replacement each month. On one day of each month, a sampling set of 14 sites was randomly chosen from a larger group of predetermined sites along the periphery of three adjacent water systems in Charleston, SC: the Ashley River (AR), Charleston Harbor (CH), and the lower Wando River (LW) (Fig. 5). The Ashley and Wando Rivers both flow into the Charleston Harbor. Each of the three water systems contained similar habitat, and were sampled using the same methods. The study included 28 AR sites (Table 1), 24 CH sites (Table 4), and 23 LW sites (Table 5), all of which were available to be selected each month. Ideally each of two boats attempted to set 12 sites in CH and LW (10 real, 2 alternates), and 14 in AR (12 real, 2 alternates) each sampling day. Weather, anglers near the sampling location, and low water levels sometimes prohibited certain selected sites from being set that day. The number of terrapins present at each site was recorded, along with several physical parameters including water salinity (refractometer), air temperature (stem thermometer), water temperature and dissolved oxygen (YSI 550A Dissolved Oxygen Instrument, YSI Incorporated, Yellow Springs, OH). The catch per unit effort (CPUE) was calculated as the number of terrapins caught at each site divided by the total number of times that site was sampled.

Statistical Analysis.---

Data collected by the SC DNR Estuarine Finfish section were used to determine if the difference in the number of terrapins caught over the fourteen-year period (1995-2009) was significantly different from zero. Since terrapins are not typically caught using trammel techniques during the cold weather, presumably due to their overwintering in the mud, all turtle data collected with water temperatures lower than 15°C were discarded. For each site and for each water system as a whole, the relationship between CPUE and year was analyzed with simple linear regression using SPSS software. Changes in water temperature and salinity, over time, were also analyzed using simple linear regression.

Land Usage.---

Data on land use change were gathered from NOAA's Coastal Services Center (CSC) Coastal Change Analysis Program (C-CAP). The following four categories of land use were considered. High intensity development is defined as having impervious surfaces over 80 to 100 percent of the total cover and includes commercial strip development, interstate highways, and runways. In medium intensity development, impervious surfaces account for 50 to 79 percent of the total cover as, for example, with many single family housing units and surrounding vegetation. In low intensity development, only 21 to 49 percent of total cover contains impervious surfaces. Open space development includes mostly vegetation with impervious surfaces accounting for less than 20 percent of total cover, for example, parks, golf courses, and vegetation surrounding more developed sites (Homer et al., 2004).

Watersheds were determined using Elevation Derivatives for National Applications (EDNA), which were derived by the U.S. Geological Survey (USGS) (Franken, 2004; Verdin et al., 2004). These watersheds outlined the areas that influenced water coming into the river systems studied. Three watersheds containing the three water systems studied were selected for analysis. ArcGIS 9 (ArcView version 9.3) was used to analyze changes in land use in the areas surrounding each watershed. Land usage changes for the three watersheds selected were mapped, and the area of change was calculated. This analysis included only changes from natural habitat to some category of development (high, medium, low or open space). The area of marsh surrounding the watershed system was measured using the area measurement tool in ArcView.

RESULTS

The Ashley River (AR) sites exhibited little change in terrapin CPUE during the study period. One site (AR17; Fig. 6) showed a significant decrease in terrapin captures over time ($p = 0.02$) (Fig. 7), while the other 27 sites showed no change (Table 6). As a whole, there was no significant change in CPUE among the AR sites ($r = 0.26$, $p = 0.26$). Of the 81.3 km^2 in the Ashley River watershed, 1.26 km^2 (2%) showed a change in land use from 1996-2006 (Fig. 8). The most common change was from natural habitat to medium intensity development.

Over the 1995-2009 period, the average salinity of the Ashley River was 20 ppt with an average temperature of 21.4 ºC. Over the same period, there has been a significant decrease ($p = 0.04$) in water temperature (Fig. 9) and there has been a significant increase in salinity ($p = 0.03$) (Fig. 10).

Little change in terrapin captures occurred in the Charleston Harbor sites. One site (CH17; Fig. 6) showed a significant decrease in CPUE during the study ($p = 0.01$), while another (CH19; Fig. 6) showed a significant increase ($p < 0.0001$) (Fig. 11). The remaining 22 sites showed no change (Table 6). Again, there was no overall change in terrapin captures for all CH sites combined ($r = 0.32$, $p = 0.24$). There has been approximately 0.714 km^2 (1% of 68.94 km^2) of land use change in the Charleston Harbor watershed from 1996-2006 (Fig. 8), predominantly from natural habitat to developed open space.

Over the 1995-2009 period, the average salinity of the Charleston Harbor was 25.2 ppt with an average temperature of 21.4 ºC. Over that same period, there has been no significant change ($p= 0.83$) in water temperature (Fig. 12) and there has been a significant increase in salinity ($p = 0.04$) (Fig. 13).

In contrast, terrapin captures decreased at several of the Lower Wando River sites. Seven sites showed significant decreases in the CPUE (LW7, LW15, LW16, LW18, LW23, LW25, and LW28; Fig. 6), while the remaining 16 sites showed no change (Table 6). Overall, the number of terrapins caught at all LW sites combined significantly decreased during the study period ($r = 0.83$, $p < 0.001$) (Fig. 14). There has been approximately 12.9 km^2 (10% of 127.72 km^2) of land use change in the Wando River watershed from 1996-2006 (Fig. 8). The most common change was from natural habitat to developed open space.

Over the 1995-2009 period, the average salinity of the Wando River was 22.05 ppt with an average temperature of 21.1 ºC. During that same period, there has been no significant change ($p = 0.18$) in water temperature in the Wando River (Fig. 15) and there has been a significant increase in salinity ($p < 0.001$) (Fig. 16).

DISCUSSION

Terrapin abundance, estimated based on CPUE, appears to have been stable in the Ashley River and Charleston Harbor during the past 15 years. The Ashley River had only one site out of 28 (3.6%) with a significant decline in terrapin catches from 1995 to 2009, and no overall decline. This specific site is located around a dock that has been present since before 1995, so it seems unlikely that its presence influenced terrapin abundance. The watershed surrounding the Ashley River sites has had minimal recent development from 1996-2006. This could be due to it being one of the oldest developed regions in the country. This lack of anthropogenic change could allow the terrapins to acclimate to the conditions of the area. They could be accustomed to normal amounts of runoff, crab fishing, boat traffic, and habitat encroachment.

The Charleston Harbor (CH) had two sites out of 24 (8.3%) with significant changes in CPUE. One showed a significant increase in terrapin catches while the other showed a significant decrease. Overall, there was no decline in terrapin abundance for the Charleston Harbor sites. The sites that experienced change in CPUE are located around Plum Island wastewater treatment plant. The wastewater is expelled deep in a shipping channel during falling tides, therefore the effects are unlikely to have been felt in the shallow water where the terrapins were caught. Changes in land use, although very small, may have influenced the outflow of the James Island Creek that could have affected the movement of terrapins from the southern CH 17 site to the northern CH 19 site, 0.5 km away. Considering all sites, the Charleston Harbor has not significantly changed in the number of terrapins caught. Like the Ashley River, this watershed experienced very little change in land use during the study period.

Unlike the other two water systems, the Wando River showed a significant decline in terrapin CPUE as well as more dramatic changes in land use. Seven of the 23 sites (30.5%) had a significant decline in terrapin catches, and there was also a significant decline for all sites combined. This area also had a much greater amount of land usage change over this period. The area that has experienced change in land use in the Wando River watershed (12.9 km^2) is at least 10 times greater than similar changes in either of the other watersheds. Several areas along the Wando River are being actively developed while the Ashley River and the Charleston Harbor are established. Thus, active development in the Wando River watershed is a possible explanation for the apparent decline in terrapin abundance in this river.

The increase in salinity for all three water systems during 1995-2009 did not seem to correspond to land use changes. These changes probably related to long term drought periods and did not seem to affect the catch per unit effort of terrapins. The significant decrease in water temperature in the Ashley River, but not in the Wando River or Charleston Harbor, also did not seem to be a cause for the change in terrapin numbers since no consistent patterns were identified. The ranges of salinity (17-29 ppt) and water temperatures (22-26 °C) during this time did not exceed the natural ranges (Dunson, 1970; Butler, 2002) where terrapins are commonly found.

A better understanding of the specific types of development in the area and how they impact the estuarine ecosystem is very much needed. It is possible that the numbers of terrapins in the Ashley River and Charleston Harbor areas were higher before active development, but unfortunately there is no documented number of terrapins in those areas during times of high development. If there was a decrease in terrapins during high development periods then the numbers became somewhat stable when active development was completed. Thus it would seem that the numbers of terrapins presently seen represent a stable population situation despite the human dominated surroundings. If this stabilization did occur, it is reasonable to think that the same thing may happen in the Wando area if development slows.

CONCLUSION

Diamondback terrapin abundance, estimated via catch per unit effort (CPUE), has remained constant for most of the Ashley River and Charleston Harbor areas from 1995-2009. Meanwhile, land use change has been minimal in both of these watersheds during this approximate time frame (1996-2006). The Wando River, on the other hand, had a significant decline in terrapin CPUE and also had a much greater amount of land use change. Land use can encroach on terrapin habitats, nesting sites and impact food and foraging areas (Ernst and Lovich, 2009). In addition, runoff and pollution due to construction could affect the water quality and forage options of the terrapin's environment. Although not a definite cause of the decline in terrapin abundance in the Wando River, land use change could greatly influence and change the habitat of the terrapin. If the numbers of terrapins in the Charleston Harbor and Ashley River in the past were affected by active land development, and subsequently became stable when the development subsided, hopefully the same trend will be seen in the Wando River area. However, until stabilization is shown, additional studies should be conducted focusing on specific causes of the decline. If the declining trend of the terrapin population in the Wando River continues, regulatory intervention or mitigation efforts may need to be considered.

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Appendix 1

The text of the advice received from ENSID is as follows :

"ENSID Technologies Ltd (ENSID) advises that rigorous post production testing has revealed a fault in a small percentage of the Integrated Circuit (IC) units used to manufacture 11mm FDX-B PIT tags supplied in batch SMT1312078. The faulty IC unit can cause a small percentage of tags from this production run, when subjected to the internal and external stresses that the tag has been designed to withstand in the fisheries environment, to fault by switching themselves on or off randomly. Although we believe this fault is only present in a very small number of the tags in batch SMT1312078, ENSID requests that Hallprint Pty Ltd (Hallprint) returns all unused 11mm PIT tags relating to this production run from the marketplace. ENSID will replace these tags with our newer "M series" 11mm polymeric PIT tag for fisheries application. The new ENSID "M series" tag is a "generation 3" tag that uses the higher powered EM4305 FDX IC unit. This new generation chip is more efficient at converting magnetic energy into signal transmission, resulting in stronger read signals. While ENSID is concerned and regrets the need to recall these tags we view this situation as an opportunity to exchange and remove older technology from the marketplace, replacing it with more efficient generation 3 tags that will help further improve detection rates of tagged fish circulating in the marine environment. ENSID will exchange all returned tags from the above batch with generation 3 tags free of charge."

Table 1. Sampling site names, latitudes, and longitudes in the Ashley River.

Site	Latitude	Longitude
AR01	32.796166674	-79.96883335
AR02	32.814499982	-79.96916669
AR03	32.798833338	-79.96966667
AR04	32.800666682	-79.97533334
AR05	32.805333328	-79.96733335
AR08	32.83	-79.97
AR09	32.830166690	-79.97116667
AR10	32.831666692	-79.97549998
AR11	32.757833354	-79.93816668
AR12	32.831333351	-79.98933334
AR13	32.831333351	-79.99166667
AR14	32.830666669	-79.99500001
AR17	32.826833344	-80.00666667
AR18	32.824833361	-80.00916667
AR19	32.825666682	-80.01533333
AR20	32.826000023	-80.01766667
AR21	32.830500031	-80.02150000
AR22	32.832333310	-80.00650000
AR23	32.839166641	-79.98866666
AR24	32.839499982	-79.98616664
AR25	32.803333346	-79.96766669
AR26	32.803666687	-79.97383334
AR27	32.807166672	-79.97266668
AR28	32.836499977	-79.99583333
AR29	32.834666697	-79.98449999
AR30	32.831499990	-79.98183333
AR31	32.810166677	-79.96449998
AR32	32.808666674	-79.96500003

Table 2. Dates of terrapin sampling in 2008 and 2009

Monday, May 05, 2008
Tuesday, May 13, 2008
Wednesday, May 14, 2008
Wednesday, May 28, 2008
Friday, May 30, 2008
Monday, June 02, 2008
Tuesday, July 01, 2008
Friday, August 01, 2008
Monday, September 15, 2008
Wednesday, October 01, 2008
Wednesday, November 12, 2008
Tuesday, March 10, 2009
Wednesday, April 08, 2009
Thursday, May 07, 2009
Monday, June 08, 2009
Tuesday, July 07, 2009
Tuesday, August 04, 2009
Thursday, September 03, 2009
Tuesday, November 03, 2009

Table 3: Initial and recapture dates, sites, PIT tag number, and sex for terrapins during 2008 and 2009 sampling seasons. Those denoted with an * are terrapins that were recaptured in a site other than the site of their original capture.

Date	Site #	Terrapin PIT #	Sex	
05/30/08	3	15609	m	
06/02/08	3		m	
05/05/08	32	16748	m	
05/28/08	32		m	
05/05/08	32	20610	f	
05/30/08	32		f	
07/07/09	32		f	
05/13/08	3	21594	m	
05/30/08	3		m	
05/13/08	11	23982	f	
06/02/08	11		f	
05/05/08	32	25712	f	
05/28/08	32		f	
05/05/08	5	26520	f	
05/13/08	5		f	
05/30/08	3	26530	f	
06/02/08	3		f	
05/14/08	3	26575	m	
05/30/08	3		m	
05/14/08	3	26762	m	
05/30/08	3		m	
05/28/08	32	26854	f	
05/30/08	32		f	
05/28/08	32	27230	m	
05/30/08	32		m	
05/28/08	12	21107	f	*
07/01/08	13		f	
05/13/08	13	27383	m	
07/01/08	13		m	
05/28/08	31	27088	m	
07/01/08	31		m	
05/05/08	26	18887	m	*
07/01/08	4		m	
07/01/08	18	25990	f	*
08/01/08	14		f	
07/01/08	31	22359	m	
08/01/08	31		m	
05/05/08	11	24578	m	
09/15/08	11		m	
05/05/08	32	21607	f	
09/15/08	32		f	
08/01/08	11	27326	f	
10/01/08	11		f	
09/03/09	11		f	
05/05/08	5	26814	f	*
10/01/08	31		f	
05/30/08	32	23942	f	*
10/01/08	31		f	
08/01/08	31	19107	f	
10/01/08	31		f	
05/28/08	5	25530	f	*
10/01/08	31		f	
08/04/09	31		f	

Date	Site #	Terrapin PIT #	Sex	
06/02/08	3	18226	m	
10/01/08	3		m	
06/02/08	23	26738	m	*
11/12/08	28		m	
07/01/08	24	22290	m	
03/10/09	24		M	
08/01/08	31	26758	m	*
03/10/09	5		M	
06/02/08	11	23557	f	*
04/08/09	10		f	
07/07/09	11		f	
05/05/08	14	27271	m	
04/08/09	14		m	
06/08/09	14		m	
05/28/08	31	27396	m	
04/08/09	31		m	
05/30/08	32	25341	m	*
04/08/09	31		m	
05/13/08	31	16704	m	
04/08/09	31		m	
05/05/08	17	26616	m	
05/07/09	17		m	
05/28/08	12	26404	f?	
05/07/09	12		f?	
05/30/08	32	18199	f	*
05/07/09	29		f	
5/13/2008	31	26399	m	*
05/07/09	32		m	
5/28/2008	32	16719	m	
05/07/09	32		m	
6/2/2008	32	21714	f	*
05/07/09	5		f	
5/13/2008	5	18257	m	
05/07/09	5		m	
8/1/2008	11	26654	f	
05/07/09	11		f	
5/5/2008	11	20444	m	
05/07/09	11		m	
5/14/2008	29	25950	f	
07/07/09	29		f	
9/15/2008	11	25637	m	
07/07/09	11		m	
9/15/2008	11	27387	m	
07/07/09	11		m	
5/28/2008	12	15264	f	*
09/03/09	14		f	
5/13/2008	13	27124	f	*
09/03/09	12		f	
07/07/09	32	122136	f	
09/03/09	32		f	
09/03/09	22	121401	f	
11/03/09	24		f	

Table 4. Sampling site names, latitudes, and longitudes in the Charleston Harbor.

Site	Latitude	Longitude
CH01	32.74783331553	-79.89333330790
CH02	32.74683335622	-79.89516665141
CH03	32.74833335876	-79.89666665395
CH05	32.75316664378	-79.89983336131
CH09	32.75316000000	-79.91494000000
CH11	32.75383332570	-79.92483336131
CH12	32.75416666667	-79.92666664124
CH13	32.75499998728	-79.92916666667
CH14	32.75566666921	-79.93183333079
CH15	32.75783335368	-79.93816668193
CH16	32.75733331045	-79.93716665904
CH17	32.76083335876	-79.94233334859
CH18	32.76166667938	-79.94466667175
CH19	32.76233336131	-79.94749997457
CH20	32.76716664632	-79.95000000000
CH21	32.76149997711	-79.94650001526
CH29	32.77533334096	-79.91333332062
CH30	32.78750000000	-79.89016666412
CH31	32.78850002289	-79.88716665904
CH33	32.78216667175	-79.88783334096
CH34	32.78183333079	-79.88700002035
CH35	32.77866668701	-79.88216667175
CH36	32.77383333842	-79.86466668447
CH37	32.77066669464	-79.86333332062

Table 5. Sampling site names, latitudes, and longitudes in the lower Wando River

Site	Latitude	Longitude
LW06	32.84116669	-79.88616664
LW07	32.84433333	-79.88750000
LW08	32.84866664	-79.88949998
LW09	32.85133336	-79.89283333
LW10	32.85445000	-79.89459000
LW15	32.86416664	-79.86983331
LW16	32.86483332	-79.86800003
LW17	32.86583335	-79.86283334
LW18	32.86583335	-79.86283334
LW19	32.86533330	-79.86100000
LW20	32.86616669	-79.86133334
LW21	32.86800003	-79.85683333
LW22	32.86916669	-79.85416667
LW23	32.87233334	-79.85066668
LW25	32.87549998	-79.84883334
LW28	32.87750003	-79.84033330
LW29	32.87916667	-79.84183331
LW30	32.88383331	-79.84366665
LW31	32.88733336	-79.84300003
LW47	32.87616666	-79.86833331
LW48	32.87549998	-79.87266668
LW62	32.87466666	-79.87733332
LW79	32.86050002	-79.88683332

Table 6. Simple linear regression R^2 values and p-values for the changes in CPUE of terrapins from 1995-2009.* denotes a significant change ($p < 0.05$) in terrapin CPUE.

Ashley River

Site	R^2 value	P value
AR01	0.04	0.465
AR02	0	0.921
AR03	0.02	0.595
AR04	0.08	0.314
AR05	0.01	0.785
AR08	1	no p value
AR09	0.02	0.603
AR10	0.03	0.550
AR11	0.03	0.530
AR12	0.06	0.399
AR13	0.04	0.450
AR14	0.01	0.664
AR17	0.34	0.022*
AR18	0.03	0.565
AR19	0.01	0.669
AR20	0.07	0.337
AR21	0.98	0.091
AR22	0	0.987
AR23	0	0.901
AR24	0.14	0.177
AR25	0.33	0.423
AR26	0.06	0.423
AR27	0.04	0.462
AR28	0.05	0.442
AR29	0.22	0.107
AR30	0.01	0.737
AR31	0.06	0.402
AR32	0	0.886

Charleston Harbor

Site	R^2 value	P value
CH01	0.25	0.083
CH02	0.06	0.406
CH03	0.1	0.247
CH05	0.04	0.485
CH09	0.12	0.771
CH11	0.04	0.457
CH12	0.04	0.491
CH13	0.35	0.095
CH14	0.02	0.637
CH15	0.15	0.156
CH16	0	0.988
CH17	0.46	0.006*
CH18	0.05	0.413
CH19	0.65	0*
CH20	0.03	0.551
CH21	0.11	0.339
CH29	0.01	0.675
CH30	0.06	0.417
CH31	0.08	0.314
CH33	0.04	0.479
CH34	0.01	0.773
CH35	0	0.838
CH36	0.2	0.144
CH37	0.21	0.111

Wando River

Site	R^2 value	P value
LW06	0.17	0.160
LW07	0.67	0*
LW08	0.24	0.062
LW09	0.06	0.397
LW10	0	0.983
LW15	0.36	0.018*
LW16	0.46	0.005*
LW17	0.03	0.604
LW18	0.48	0.004*
LW19	0.06	0.393
LW20	0	0.844
LW21	0.01	0.699
LW22	0.12	0.208
LW23	0.54	0.002*
LW25	0.54	0.002*
LW28	0.26	0.054*
LW29	0.15	0.157
LW30	0.08	0.371
LW31	0.01	0.790
LW47	0.06	0.385
LW48	0.08	0.316
LW62	0.01	0.793
LW79	0	0.964

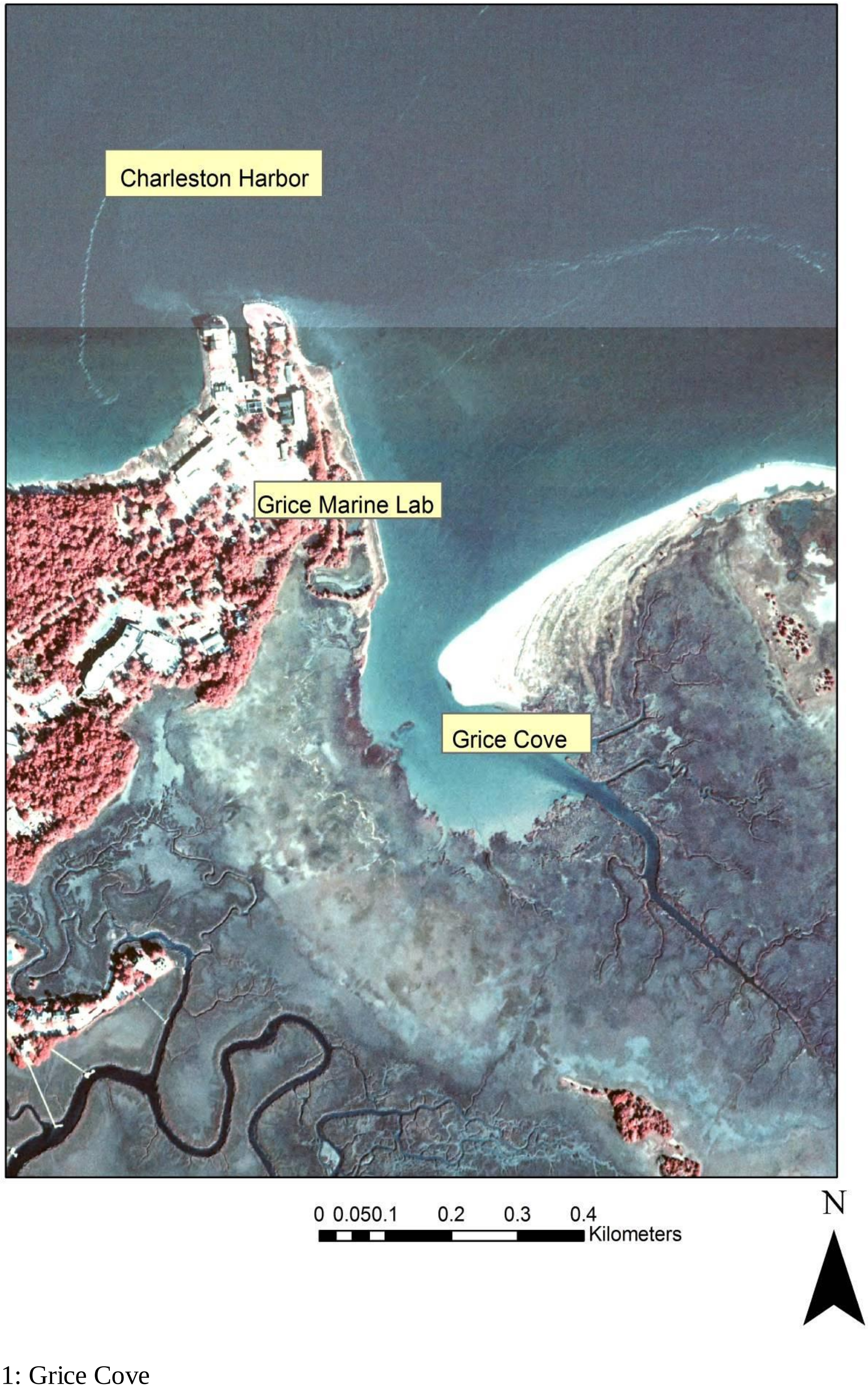

Figure 1: Grice Cove

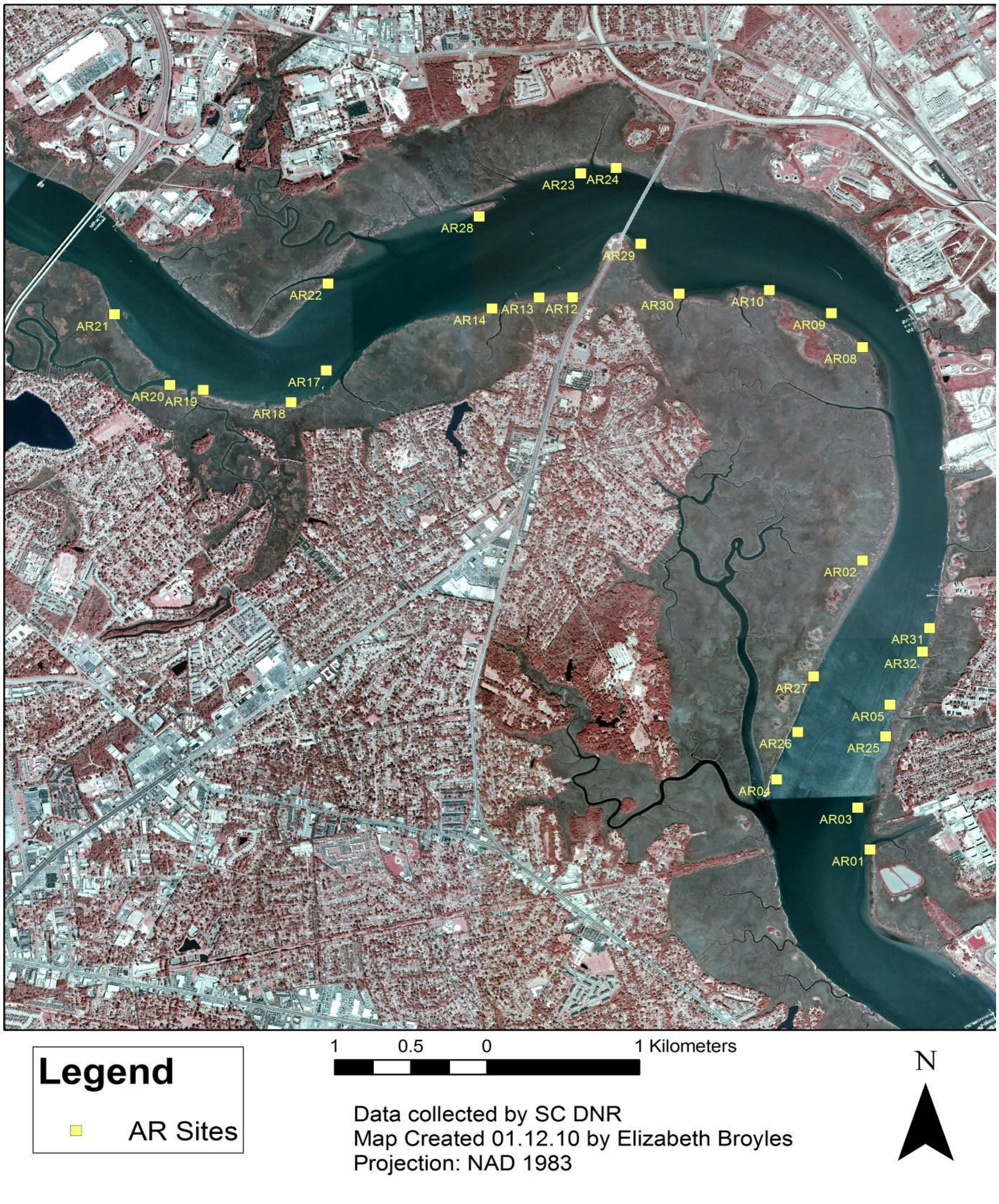

Figure 2. Sites along the Ashley River, Charleston, SC sampled for terrapins during the 2008-2009 seasons.

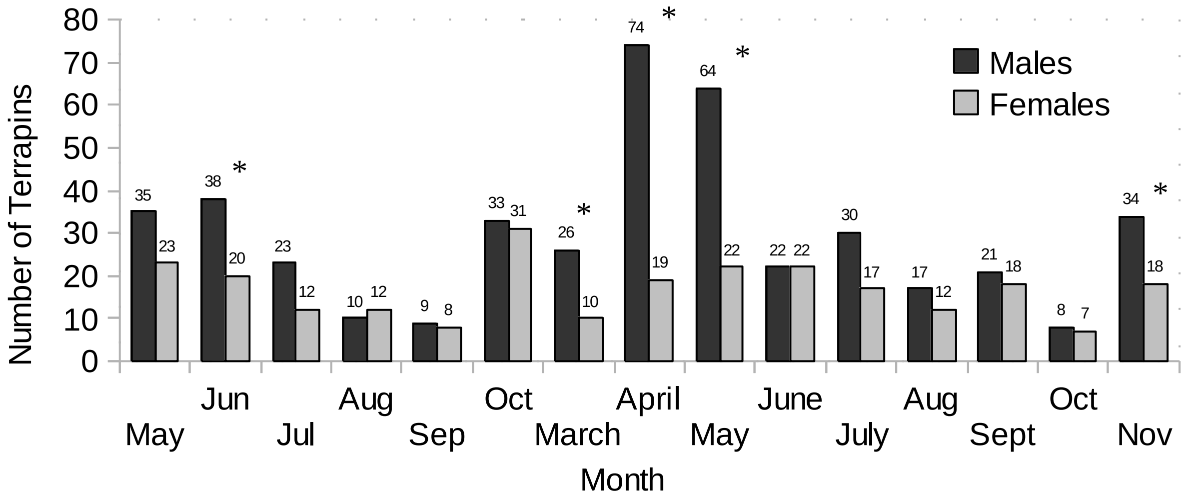

Figure 3. The sex ratio of terrapins captured in the Ashley River from 2008 to 2009.
* indicates a significant difference of $p < 0.05$.

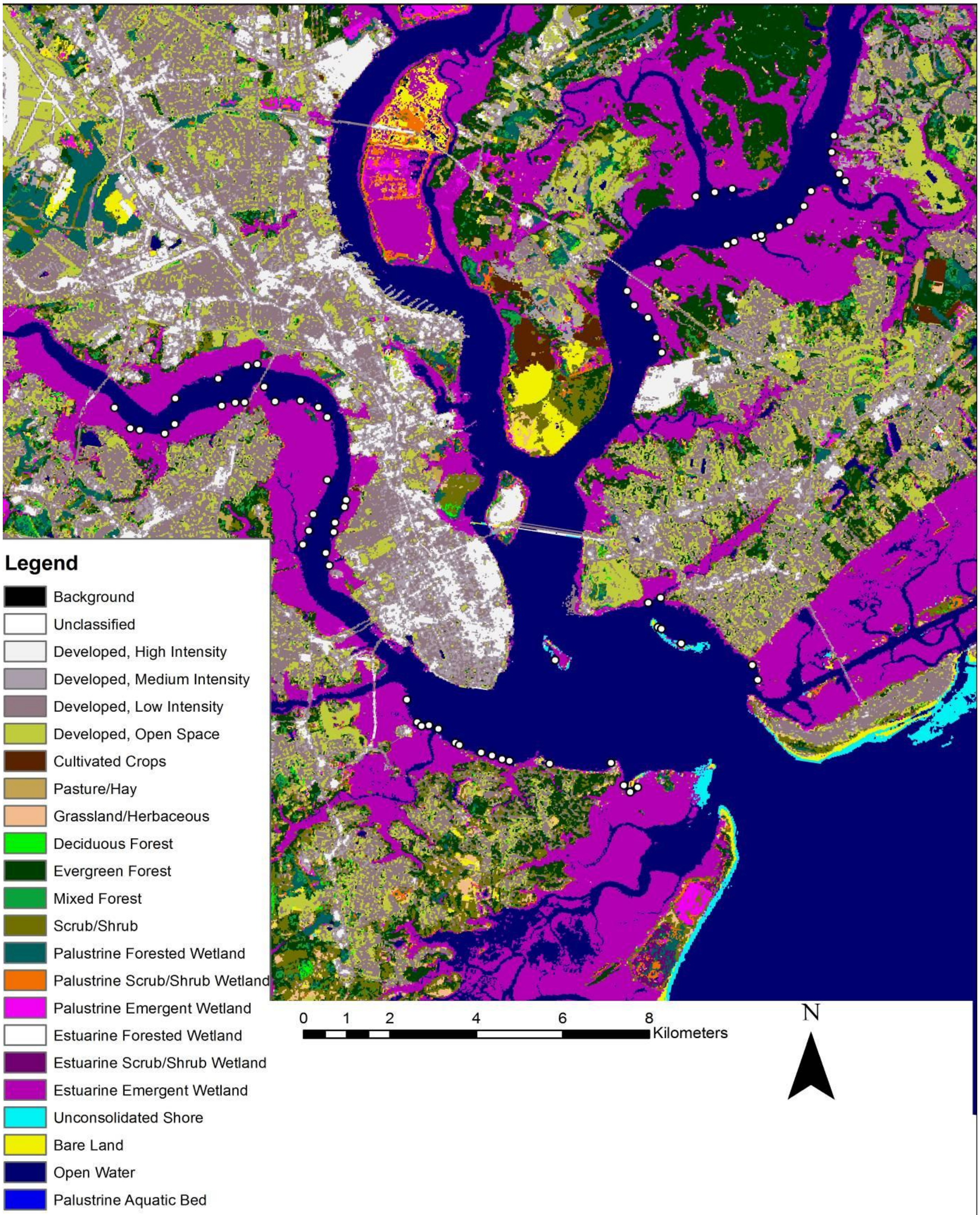

Figure 4. Land usage for the Charleston SC area including the sampling sites from AR, CH and LW water systems.

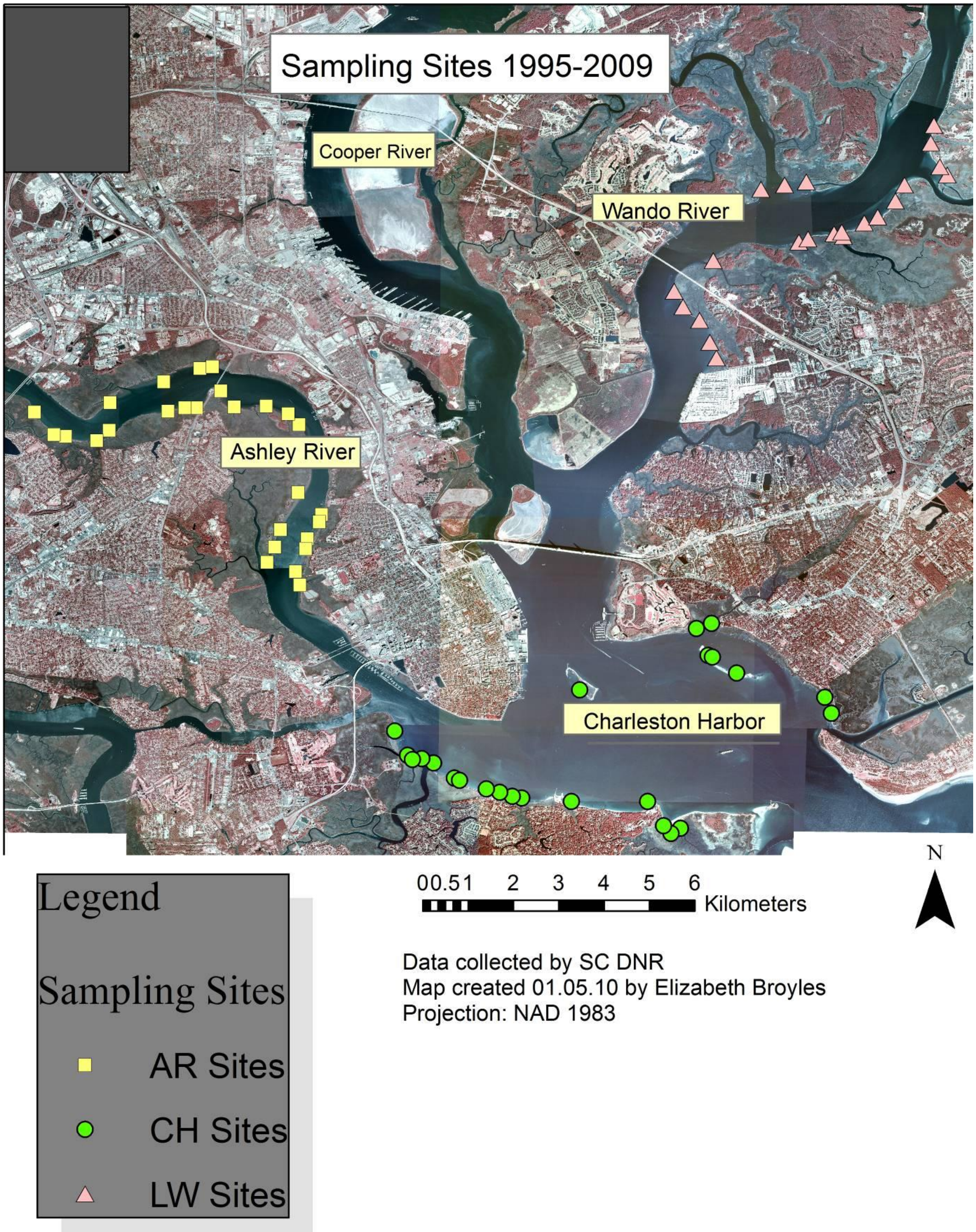

Figure 5. Sampling sites for the Ashley River, Charleston Harbor and lower Wando River.

Figure 6. Sites with a significant change in CPUE of terrapins from 1995-2009.

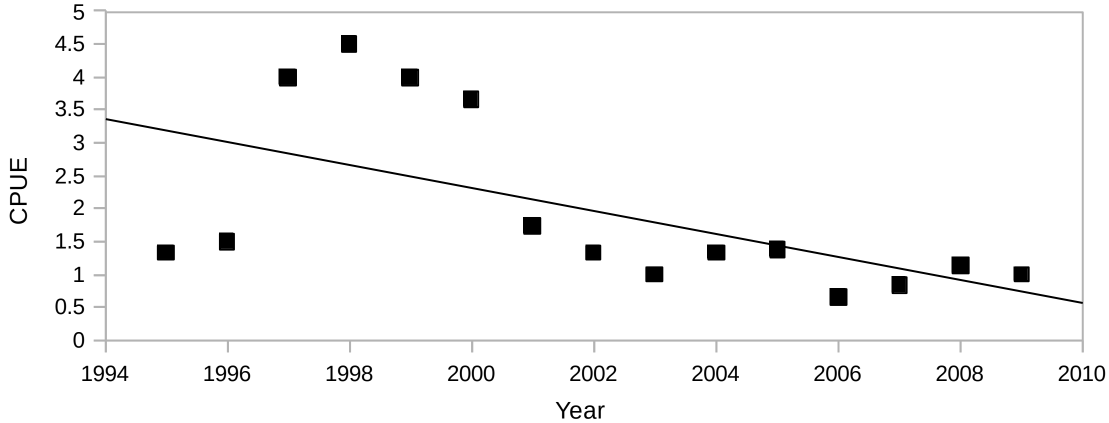

Figure 7. Catch per unit effort of terrapins caught at site AR17 in the Ashley River from 1995 to 2009.

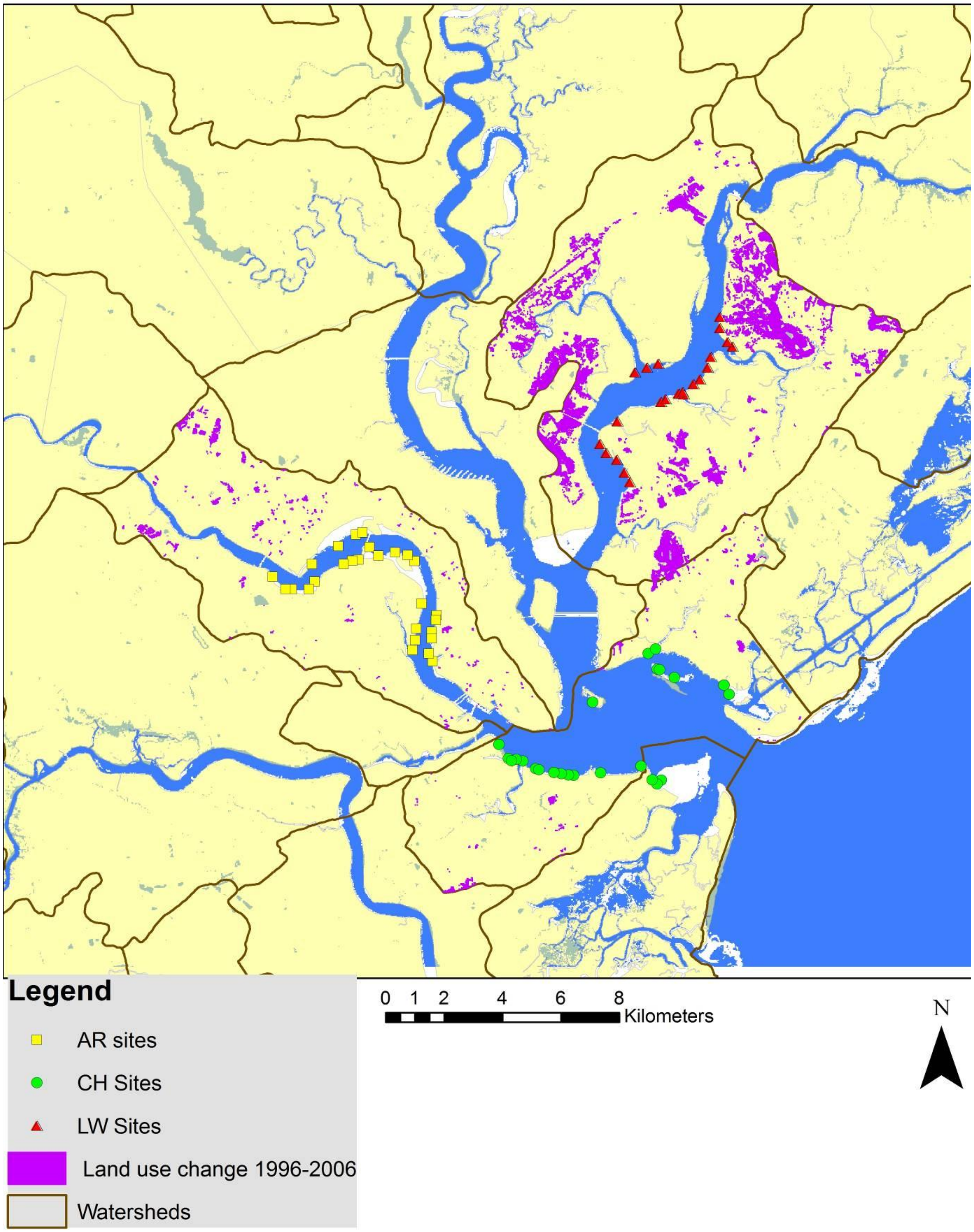

Figure 8. Land usage change from 1996- 2006 in the watersheds containing the Ashley River, Charleston Harbor and lower Wando River sampling sites.

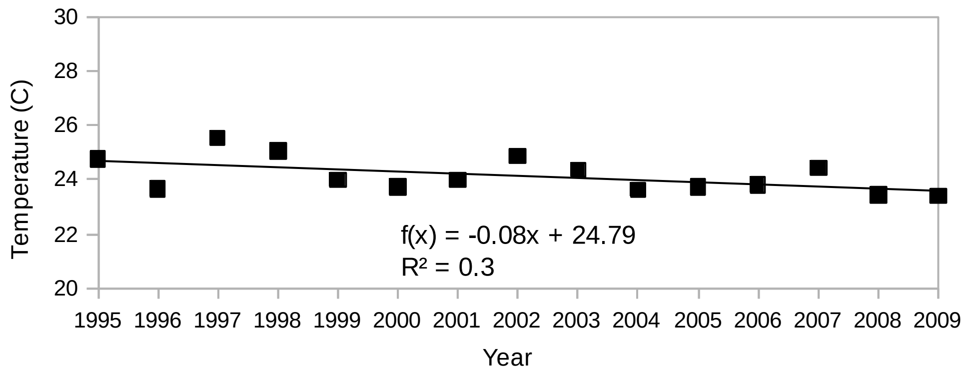

Figure 9: Ashley River average water temperature from 1995-2009

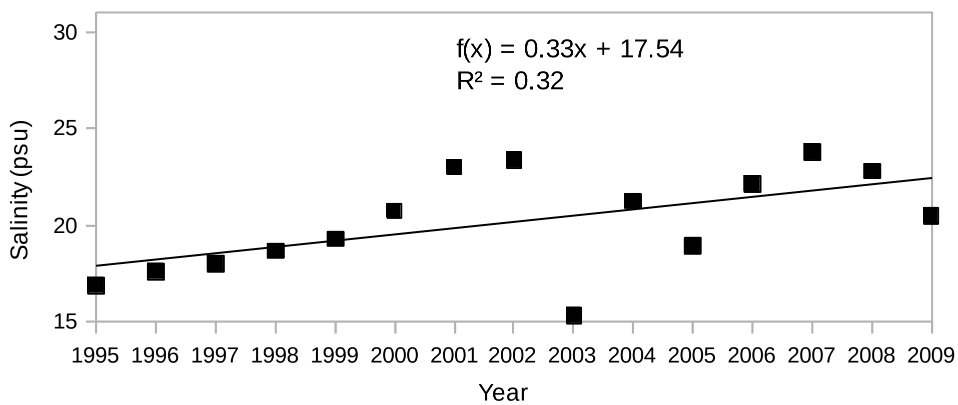

Figure 10: Ashley River average salinity from 1995-2009

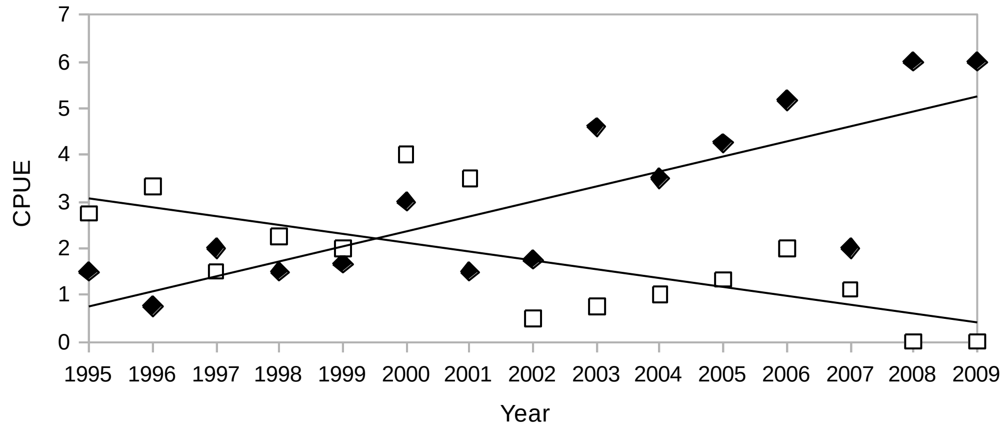

Figure 11. Catch per unit effort of terrapins caught at sites CH17 (white square) and CH19 (black diamond) in the Charleston Harbor from 1995 to 2009.

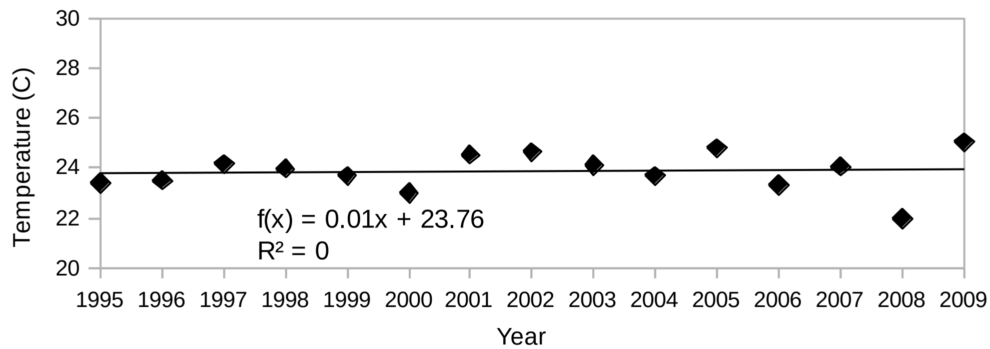

Figure 12: Charleston Harbor average water temperature from 1995-2009

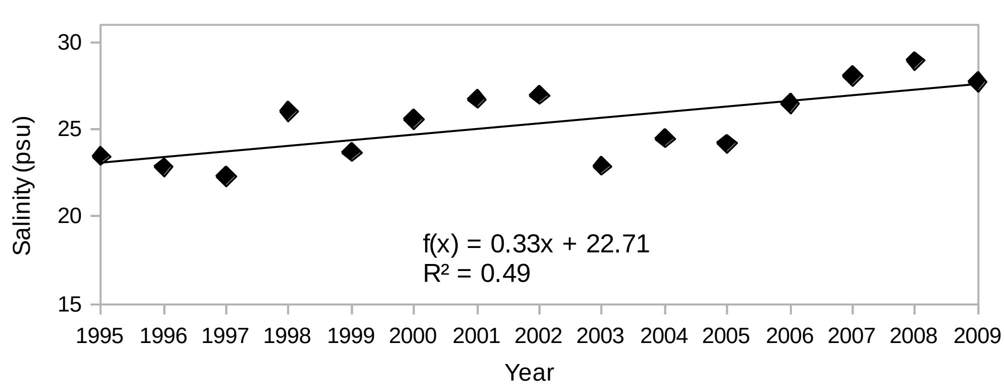

Figure 13: Charleston Harbor average salinity from 1995-2009

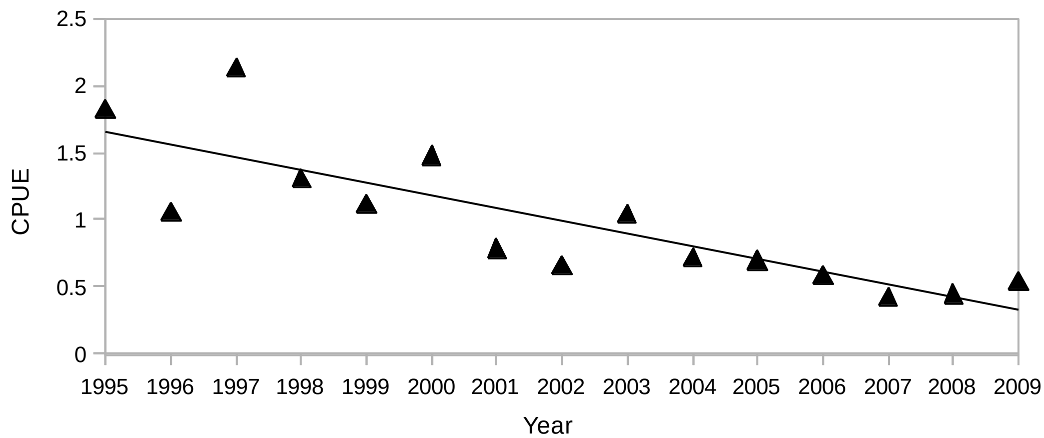

Figure 14. Catch per unit effort of terrapins caught at all sites in the lower Wando River combined from 1995 to 2009.

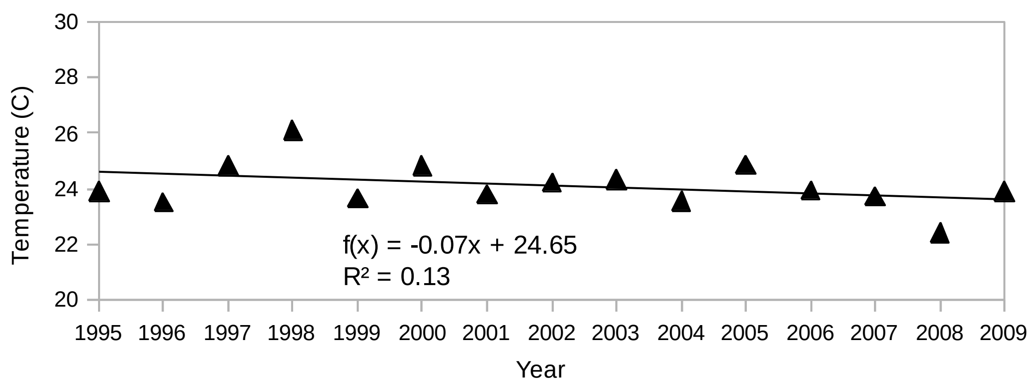

Figure 15: Wando River average temperature from 1995-2009.

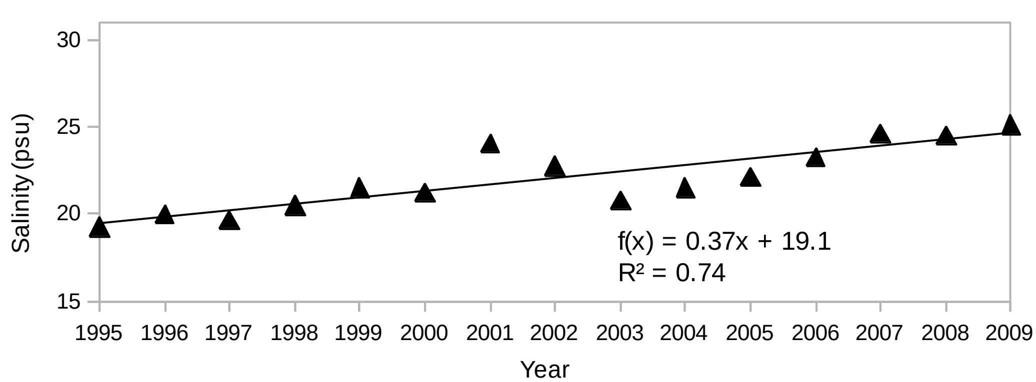

Figure 16: Wando River average salinity from 1995-2009