Mating system and dispersal patterns in the diamondback terrapin (*Malaclemys terrapin*)

A Dissertation
Submitted to the Faculty
of
Drexel University
by
Claire Marie Sheridan
in partial fulfillment of the
requirements for the degree
of
Doctor of Philosophy
April 2010

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank all those whose abilities, efforts, facilities or funds allowed the completion of this project. First, I would like to thank my husband, Michael, for all of his love and support over the past 5 years. Without him, I would not have made it this far. I would like to thank the rest of my family, especially my mother and my parents-in-law, for all the emotional support that they have provided me.

I thank my colleagues and friends in Drexel's Biology Department: John Wnek for all the great discussions and help in selecting field sites; Jules Winters, Lori Lester, Abby Dominy, Karen Sullam, and Emily Basile for all hard work you helped me to accomplish and the good times that came along with it; Steve Pearson and Julia Stone for always being there to listen to me ramble on in the office; and Laurie Cotroneo for being there as a great friend and the one person with whom I could talk about genetics. I am also grateful to Ashley Lavender who has been a great listener and friend, regardless of moving miles away from Drexel.

I am grateful to the other people who helped me in the field: Earthwatch volunteers, MATES students, Chaz Sineri, Sam Campos, Bryan Vorbach, Julie Fekete, Chris Wilson, and Mallory Watson. I am grateful to Jacquie Walters, Andy Harrison, and Dr. Ed Standora from Buffalo State for helping me during various field seasons. I am also grateful to James Houck for helping me in the lab. I thank Dr. Willem Roosenburg for his willingness to collaborate, facilitating sample collection at Poplar Island, and critically reviewing the mating system manuscript.

I am very grateful to my advisors, Dr. Harold Avery and Dr. James Spotila for the years of guidance they have given me. They have both been great mentors and friends. I'll never forget our many great field adventures. I am grateful to Dr. Walter Bien for his advice throughout the years and for showing me around the Pine Barrens. I would also like to thank the rest of the members of my committee, Dr. Kim Scribner, Dr. Jacob Russell, and Dr. Shivanthi Anandan for providing invaluable comments, suggestions, and thought provoking questions throughout my dissertation project.

This research was funded by the Earthwatch Institute and the Betz Chair of Environmental Science at Drexel University. Scientific Collecting and Holding Permits were issued yearly by the NJDEP Division of Fish and Wildlife and Special Use Permits were issued yearly by the Edwin B. Forsythe National Wildlife Refuge. Research protocols were approved by Drexel University's Institutional Animal Care and Use Committees.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Mating system and dispersal patterns in
the diamondback terrapin (*Malaclemys terrapin*)
Claire Sheridan
Harold W. Avery, co-Supervisor, Ph.D.
James R. Spotila, co-Supervisor, Ph.D.

Long-term demographic studies of the diamondback terrapin (*Malaclemys terrapin*) indicate high habitat fidelity and low dispersal distances, but population genetic studies indicate high levels of gene flow between populations. In addition to conflicting data between direct and indirect measurements of dispersal in the terrapin, data are currently lacking on how habitat fragmentation affects the dispersal and mating system of the terrapin. Dispersal and mating systems are important to understand because they can affect the level of genetic diversity in a population, and therefore influence its long-term sustainability of a population. In this study, I examined the mating system and fine-scale dispersal patterns of diamondback terrapins in Barnegat Bay, New Jersey. Using both capture-mark-recapture and molecular genetic methods, I compared dispersal patterns of juvenile, male, and female terrapins in Barnegat Bay, NJ. While capture-mark-recapture over a four year study period indicated that all individuals have relatively small dispersal distances (<2 km), mean genetic assignment indices, first generation migrant tests, and spatial autocorrelation indicated that mature males exhibited sex-biased dispersal and mature females exhibited natal philopatry to nesting beaches. To determine how habitat fragmentation affects dispersal and gene flow in the terrapin, I used a landscape genetic approach. Landscape genetic models indicated that estuarine emergent

wetland was a landscape feature necessary for effective dispersal and therefore necessary to maintain genetic diversity in the terrapin. Because mating systems can also affect the level of genetic diversity, I also analyzed paternity in 174 nests from five nesting beaches. Multiple paternity was common in nests, the degree of multiple paternity differed significantly among locations, ranging from 12.5 to 45.7 % of clutches, and exhibited a significant non-linear correlation with population sex ratio. Population sex ratios were likely altered by differential mortality of nesting females on roads or of males in commercial crab pots. I confirmed the use of sperm storage both within and between seasons, but found no data to support long-distance movements of individuals to mating aggregations. Utilization of genetic methods has yielded important information regarding terrapin dispersal and mating behaviors that is particularly important in developing conservation and management plans. The data indicate that protection of estuarine emergent wetland and natal nesting beaches is necessary for species survival, and that differential mortality, resulting in skewed sex ratios, can have significant impacts on the mating system of *Malaclemys terrapin*.

CHAPTER 1: Introduction

1.1 Habitat fragmentation decreases biodiversity

Habitat alteration represents the primary driving force in the loss of biological diversity worldwide (Vitousek *et al.* 1997). Anthropogenic habitat alteration and degradation are estimated to have altered 39-50% of land on earth (Daily 1995; Vitousek *et al.* 1986). However, these values underestimate the impact of habitat transformation because they do not include the habitat that becomes fragmented when surrounding habitat has been altered (Vitousek *et al.* 1997). The effects of habitat fragmentation on animal populations have been documented extensively (Fahrig 2003; Laurance & Bierregaard 1997; Prugha *et al.* 2008; Whitcomb *et al.* 1981). Habitat fragmentation can increase the rate of population extinction through genetic (Saccheri *et al.* 1998) and demographic factors (Boudjemadi *et al.* 1997). However, the effects of habitat fragmentation on an animal's behavior, such as dispersal and mating, are not as well understood (Stow & Sunnucks 2004a, b; Stow *et al.* 2001). Habitat fragmentation may restrict dispersal and reduce social neighborhood sizes; these changes may influence the mating system (Cale 2003; Stow & Sunnucks 2004b). For example, if habitat fragmentation leads to delayed or impeded dispersal, then this change in dispersal could lead to increased competition, elevated philopatry, and disruption of adaptive kin structures and breeding opportunities (Bjørnstad *et al.* 1998; Boudjemadi *et al.* 1997; Peacock & Smith 1997a; Stow *et al.* 2001).

1.2 Estuaries as important ecosystems

Estuaries are among the most productive ecosystems in the world and they provide critical resources for birds, fish, and other wildlife (McLusky & Elliot 2004). One such estuary is Barnegat Bay, a 70 km long estuary located along the central coast of New Jersey. Barnegat Bay is ecologically threatened by changes in water quality and quantity, habitat loss and alteration, fisheries decline, and other induced changes. The region has experienced anthropogenic changes for at least 350 years, since the first Europeans settled in the mid 17th century (BBNEP 2002). Over 450,000 people live within the watershed, but land and water use doubles during the summer vacation months (BBNEP 2002). At least 45 percent of the shoreline is bulkheaded and nearly 70 percent of the shoreline buffer zone is either developed or altered (BBNEP 2002).

The United States Environmental Protection Agency listed Barnegat Bay as an estuary of national significance and developed a Comprehensive Conservation and Management Plan (CCMP) for the region. The CCMP outlined priority problems and solutions needed to restore and protect the ecological health of the estuary (BBNEP 2002). The CCMP was divided into the following four major action plans (and further subdivided into action items): 1. Water Quality/Water Supply (25 Action Items), 2. Habitat and Living Resources (12 Action Items), 3. Human Activities and Competing Resources (10 Action Items), 4. Public Participation and Education (20 Action Items; (BBNEP 2002).

One action item (Action Item 6.2 under the Action plan: Habitat and Living Resources) outlined in the BNEP-CCMP was to conduct a Barnegat Bay ecosystem restoration feasibility study (BBNEP 2002). This feasibility study considered the following areas for restoration to improve habitats for numerous species of plants and animals: fresh-water wetlands, salt marshes, abandoned lagoons, and submerged aquatic vegetation (BBNEP 2002). The feasibility study was conducted as a joint investigation between the United States Army Corps of Engineers and the New Jersey Department of Environmental Protection and was completed in 2003 (United States Army Corps of Engineers 2003). Six priority sites were chosen for immediate evaluation (United States Army Corps of Engineers 2003). Restoration of several of these sites has the potential to improve both aquatic habitat and terrestrial habitat of *Malaclemys terrapin*. For example, one tidal marsh site at the northernmost part of Barnegat Bay, named the F and L abandoned lagoons, will be restored in part by filling in the previously dredged lagoons to create a maximum water depth of 1.83 meters (6 feet) and in part by improving circulation by adding a new tidal connection. Both restoration plans would allow for the growth of submerged aquatic vegetation (SAV) and improved water quality, increasing habitat for fish, benthic invertebrates, and *Malaclemys terrapin.* In addition, sandy piles within this site will be cleared and flattened to provide nesting habitat for *Malaclemys terrapin* (United States Army Corps of Engineers 2003).

In the 2003 Monitoring Program Plan, the Barnegat Bay National Estuary Program established a series of environmental and programmatic indicators to

evaluate the progress of the action items in the CCMP (BBNEP 2003). Nine primary indicators and twenty-one secondary indicators were chosen. Indicators were discussed in relation to habitat, water resources, and human use. Primary indicators fit three criteria: 1. public acceptability, 2. availability of data from existing monitoring efforts, 3. relevance to the goals and objectives of the CCMP. Signature species are listed as one of the nine primary indictors. Signature species are those that the public identifies with the Barnegat Bay watershed. Signature species were selected by choosing species which represent the range of habitats that comprise the Barnegat Bay system. These habitats include terrestrial, freshwater, and estuarine habitats. By tracking a signature species in each of the these habitats, BBNEP determines which habitats are in need of restoration and which habitats are benefiting from the improvements that have already been made (BBNEP 2003). Although, the BBNEP derived the list of signature species from avian species, anuran species, finfish species, estuarine macroinvertebrates, and terrestrial plant species (BBNEP 2003), reptilian species should also be considered.

1.3 The diamondback terrapin as a model vertebrate

The diamondback terrapin, *Malaclemys terrapin*, can be used as an indicator of the ecological function of the estuary. The diamondback terrapin is a species found locally within the salt marshes of the eastern and Gulf coasts of the United States (Ernst *et al.* 1994). Of over 310 extant species of turtles, *Malaclemys terrapin* is the only species whose habitat is confined to coastal brackish waters (Rhodin *et al.*

2009). Diamondback terrapins are euryhaline and can tolerate a wide range of water salinities from 0 to 34 ppt (Robinson & Dunson 1976). Although terrapins have salt glands, after an extended period of time in salinities above 21 ppt they require some source of freshwater (Dunson & Mazzotti 1989). The diamondback terrapin occupies a high trophic level and consumes polychaetes, shrimp, littorinid gastropods, bivalves, fish, and crabs (Carr 1952; Coker 1906; Davenport *et al.* 1992; Hurd *et al.* 1979; Petrochic 2009; Tucker *et al.* 1995). The diamondback terrapin may exert top-down control on the grazer density of the periwinkle snail, *Littoraria irrorata*, which grazes upon salt marsh cordgrasses such as *Spartina alterniflora* (Levesque 2000; Silliman & Bertness 2002) or it may help maintain higher species diversity by consuming large quantities of various species and not allowing any one species to dominate (Petrochic 2009). Furthermore, due to its high trophic level, bioaccumulation of mercury in the liver tissue of the diamondback terrapin inhabiting Barnegat Bay, NJ is sufficiently high enough to cause sublethal effects on scavengers (Burger 2002). The diamondback terrapin is a habitat generalist that utilizes both the terrestrial and aquatic habitat of an estuary for foraging, mating, and nesting. Because this species is a food (Petrochic 2009) and habitat generalist, it can be used as an indicator of the ecological function of the estuary, particularly in response to anthropogenic impacts on both land and in the water. These impacts may include dredging and filling of marsh habitat, human development on terrestrial nesting habitat, bulkheading along the edges of terrestrial nesting and marsh habitat, and boat traffic in aquatic habitats.

1.4 Background on the diamondback terrapin

Diamondback terrapins are small turtles that exhibit strong sexual size dimorphism, with females significantly larger than males at reproductive maturity. Mean plastron length of male *M. terrapin* is 10.2 cm and of females is 14.8 cm (Lovich & Gibbons 1990; Seigel 1984; Tucker *et al.* 1995). Both males and females have life spans of greater than 20 years, but males mature earlier than females. Males are recruited into the adult population at approximately 4 to 7 years of age while females reach maturity between 8 to 13 years of age (Roosenburg 1990). Because male and female terrapins reach different sizes at sexual maturity, adult male and female feeding niches have minimal overlap (Tucker *et al.* 1995). Males and small females tend to choose smaller prey items, such as the salt marsh periwinkle (*Littorina* irrorata*).* Large females feed on larger prey items such as the fiddler crab (*Uca pugnax*) and the blue crab (*Callinectes sapidus*; (Levesque 2000; Tucker *et al.* 1995).

The annual activity cycle of the terrapin varies among areas due to its wide latitudinal distribution (Ernst *et al.* 1994; Hildebrand 1928; Yearicks *et al.* 1981). In the winter, terrapins hibernate individually or in groups buried in creek banks and bottoms (Yearicks *et al.* 1981). Terrapins can briefly emerge from hibernation on warm winter days (Yearicks *et al.* 1981), but it is not known whether feeding occurs during this period. In New Jersey, the terrapin (subspecies *M. terrapin terrapin*) hibernates from November to March and is typically active from April to October, with nesting occurring from late May-mid July (Wood & Herlands 1997).

The diamondback terrapin has a type III survival pattern (*sensu* Iverson 1991) with low juvenile survival followed by high adult survivorship. Therefore, in terms of population viability, adult survival and fitness are extremely important in maintaining a population. Many anthropogenic actions lower survival rates or fitness of adult terrapins causing populations to decline. Diamondback terrapins were a delicacy in the late 1800s and early 1900s (Carr 1952) and populations were severely depleted. Besides exploitation as a food source, diamondback terrapins also face other anthropogenic threats: loss of nesting locations (Szerlag & McRobert 2006; Wood & Herlands 1997), incidental capture and mortality in crab pots (Bishop 1983; Roosenburg *et al.* 1997; Seigel 1983; Tucker *et al.* 2001; Wood 1997), degradation or loss of aquatic habitat (Wood & Herlands 1997), and mortality on roads (Szerlag & McRobert 2006; Wood & Herlands 1997). In addition, many states list the protection or game status of this species differently. The listings range from no official listing, game listing, Species of Special Concern, Threatened, to Endangered (Watters 2004). New Jersey lists the terrapin as a Species of Special Concern, but also as a game species (N.J.A.C. 1981). New Jersey lists species under the title Species of Special Concern if there is some indication of population decline or indication of vulnerability due to habitat degradation or modification that might lead to the population being listed as Threatened in the future. In addition, a species may also be listed under this title if there is not enough information about its current population status. In New Jersey, terrapins may be caught between November 1 to March 31 if

they are over 5 inches (152 mm) in length, but no person is allowed to take or destroy eggs of any terrapin (N.J.A.C. 1981)

1.5 Terrapin dispersal patterns measured by direct analysis

Nesting ecology and population dynamic studies have documented that terrapins exhibit high fidelity to nesting sites (Auger 1989; Mitro 2003; Roosenburg 1996) and to specific creeks or sections of a river (Gibbons *et al.* 2001; Hurd *et al.* 1979; Roosenburg *et al.* 1999; Spivey 1998). Male and female terrapins stay within a few meters of their original capture locations for up to three years (Lovich & Gibbons 1990); similar results were seen after 16 years (Gibbons *et al.* 2001). In New Jersey, female terrapins often return to nest year after year to the same dune area in Little Beach, NJ (Burger 1977). Another study in Tuckerton, New Jersey (Szerlag & McRobert 2007) found that 33-40% of females were found within 50 m of the original site over two nesting seasons (includes multiple clutching and multiple seasons). As a consequence of high fidelity, it is believed that rates of exchange among subpopulations are unlikely to lead to the reestablishment of adjacent populations that are extirpated (Tucker *et al.* 2001).

Some studies have recorded occurrences of longer distance movements of terrapins. Butler (unpublished) tracked the movements of three females located on a nesting beach and found that they traveled between 4.8 and 9.98 km to marsh areas, but then spent the rest of the year in these locations. Gibbons et al. (2001) reported a round-trip distance of 5.5 km for one female from a creek to a nesting site and back.

Although nesting female terrapins are occasionally reported to travel long distances to nesting beaches, these events seem to be rare rather than the norm.

Home range analysis of radio-transmittered terrapins revealed mean home ranges of 0.54 km^2 in northeastern Florida (n=8) and 3.05 km^2 in North Carolina (n=10; both values represent Minimum Convex Polygon, 95% isopleths; Butler unpublished; Spivey 1998, respectively), with home ranges including frequently flooded low marsh (Spivey 1998). Spivey (1998) suggested that over 700 km of artificial ditching may have expanded terrapin home ranges in South Carolina by allowing longer straight-line movements, connecting foraging centers and increasing the number of activity centers, or causing some areas to be suboptimal and requiring larger home ranges to meet terrapins' needs.

1.6 Terrapin dispersal patterns measured by genetic analysis

Studies utilizing mitochondrial DNA (mtDNA) found low levels of genotypic diversity along the range of *Malaclemys terrapin* (Hauswaldt 2004; Lamb & Avise 1992) indicating the possibility of high levels of gene flow facilitated by dispersal. One pattern that was consistent among both studies of mtDNA was that terrapins sampled from Texas to Cape Canaveral, Florida were distinguished from terrapins north of Cape Canaveral, regardless of whether the mtDNA was analyzed with restriction fragment length polymorphism (Lamb & Avise 1992) or by sequencing (Hauswaldt 2004).

Microsatellite DNA generally reveals greater diversity than many other molecular techniques for measuring genetic diversity because of the high mutation rate and co-dominant inheritance of microsatellite DNA. Microsatellites have a higher mutation rate, 10^{-3} to 10^{-4} per locus per replication, compared to the frequency of point mutations, 10^{-9} to 10^{-10} (Lowe *et al.* 2004). Consequently, they can exhibit high number of alleles per locus, which allows for calculation of high exclusion probabilities and the potential for identifying private alleles (Lowe *et al.* 2004). Using six highly polymorphic microsatellite loci, Hauswaldt (2004) calculated Wright's fixation index, F_{ST}, (Wright 1978) a widely used measure of genetic differences among populations. Utilizing qualitative guidelines established by Wright (1978), Hauswaldt (2004) found no data for genetic differentiation within seven sampling locations in the Charleston Estuary (SC), with maximum distances between sampling sites up to 30 km (N=130). Hauswaldt (2004) suggested that this lack of genetic structure may be caused by different life history events that are not typically recorded in nesting or habitat utilization studies: 1. male and female terrapins moving to mating aggregations during the spring, but afterwards return to their home sites or 2. juvenile dispersal.

In 2005, Hart hypothesized that gene flow among populations would be low due to high site fidelity. Hart (2005) measured pair-wise genetic distance values (D_{CE}) and Wright's fixation index (F_{ST}) in terrapins from 31 sites along the entire range of the terrapin from Massachusetts to Texas using twelve polymorphic microsatellite markers. Genetic distance values are greatest between the two extremes of the

Atlantic portion of the range [D_{CE} =0.71 and F_{ST} = 0.476 between Barnstable, MA (N=19) and Nest Key, FL (N=13), geographic distance=2,244 km; Hart 2005]. Genetic distance values are lowest when comparing locations sampled within the same estuary (eg. D_{CE} =0.09 and F_{ST} = 0.001 Big Sable Creek, FL (geographic distance =1 km, N=101, N=133) but moderate when comparing those within a region (eg. D_{CE} =0.24 and F_{ST} =0 .016 between Sandy Hook, NJ (N=22) and Cape May, NJ (N=29), geographic distance=169 km). When comparing genetic distance values with actual distance between the sample locations, the pattern follows an isolation-by-distance model of population structure (Hart 2005). Using the genetic distance values in a neighbor-joining phenogram, there was evidence for a high degree of regional differentiation among the sampled locations (Hart 2005). This allows establishment of six genetically-based management units. Each management unit is composed of one regional location: Gulf Coast, South Florida, Coastal Carolina, Chesapeake Bay, Coastal Mid-Atlantic, and Northeast Atlantic. Gene flow in the Florida Everglades and North Carolina (study locations consisted of continuous, undeveloped salt marsh coastline; Hart, personal communication) is due to movement of males (Hart 2005), which is called male-biased dispersal (based on Mean Assignment Indices). However, movement of males to an extirpated area will not allow for recolonization of that area, unless females also move into that area. Hart (2005) concluded that genetic differentiation and population genetic structure exists at all hierarchical levels. Although strong evidence for site fidelity in exists in demography studies (Gibbons *et*

al. 2001), lowered genetic differentiation within regional locations suggests significant gene flow within regional locations (Hart 2005).

1.7 Effects of habitat fragmentation on mating behavior

Habitat fragmentation in some species reduces dispersal and causes loss of genetic diversity within habitat patches and an increase in differentiation among habitat patches (Sarre *et al.* 1990). The effects of habitat fragmentation on an animal's behavior and genetics within a habitat patch have not been well studied (Stow *et al.* 2001). For example, habitat fragmentation could affect mating systems (Peacock & Smith 1997a, b; Stow & Sunnucks 2004a, b; Stow *et al.* 2001). Smaller habitat patches or suboptimal habitats may reduce population densities or alter sex ratios due to differential mortality caused by human interactions (e.g. crab pot mortality and road mortality). Changes in population density and sex ratios may affect the occurrence of monogamy, polyandry, and polygyny by altering the density of mating aggregations and/or strength of competition for mates (Kamler *et al.* 2004; Mobley & Jones 2007; Soucy & Travis 2003; Westneat & Sherman 1997). The availability of acceptable mates may also decrease the mating system's effective population size (N_e; Sugg & Chesser 1994). The effective size of a population, N_e, is the size of an ideal population that would maintain genetic variability, due to random processes, at the same level as the actual population (Lande & Barrowclough 1987; Wright 1931). An ideal population is characterized by random mating, constant population size, no variance in reproductive success, and equal numbers of males and

females (Hartl 1988). In an ideal population, N_e is equal to N, the census population size (Hartl 1988). In actual populations, N_e is almost always less than N, and this difference may be due to several causes, including reproductive success, the mating system, or population sex ratios (Hartl 1988).

Mating systems in which a female mates with more than one male, polyandry, can increase the N_e above the N_e of mating systems with only monogamy or polygyny (Olsson *et al.* 1994; Sugg & Chesser 1994). Therefore, polyandry has been suggested to decrease inbreeding (Hoske & Blanckenhorn 1999; Petrie & Kempenares 1998; Tregenza & Wedell 2000). Both inbreeding and mate limitation can increase a population's risk for extinction because they reduce the N_e and decrease population fitness (Keller & Waller 2002; Saccheri *et al.* 1998; Young *et al.* 2000).

1.8 Diamondback terrapin mating behavior

To date, few researchers have intensively studied mating in the terrapin in either its natural environment (Hauswaldt 2004) or in captivity (Hildebrand 1928). Courtship and mating are thought to occur in early spring, shortly after emergence from hibernation, and are followed by nesting in late spring (Seigel 1980; Zimmerman 1989). Mating aggregations have been documented, in which 6-75 male and female turtles were observed (Seigel 1980). However, mating behavior and success are difficult to observe because mating aggregations occur in the murky, turbid waters of the marsh. More recently with the use of bi-parentally inherited DNA, researchers may determine the outcome of the mating behaviors of the terrapin

without the need for visual observation. Previous studies have documented that terrapins in captivity have the ability to produce a high percentage of fertile eggs for at least two years without recopulation (Hildebrand 1928), implying that they have sperm storage capabilities. In addition, terrapins are thought to engage in a polygamous mating system, which may complicate investigations of operational vs. apparent sex ratios (Roosenburg *et al.* 1997). While apparent sex ratios often vary among different study populations, such as Merritt Island, Florida 5:1 female biased (Seigel 1984) and Kiawah Island, South Carolina, 1:1.78 male biased (Lovich & Gibbons 1990), the sex ratio necessary to produce 90% fertility in eggs in captivity appears to be about one male to five females (Hildebrand 1928). Despite the fact that mating systems are likely to vary over space and time (Jones *et al.* 2001), the mating system of the diamondback terrapin has only involved one example population during one nesting season. Hauswaldt (2004) estimated that polyandry, the occurrence of multiple mating in females, occurred in 18% of clutches in Oyster Bay of Long Island, New York. However, polygyny was not documented, indicating the possibility of a large breeding population (Hauswaldt 2004). Hauswaldt's study population had a sex ratio of one to one (Hauswaldt 2004).

1.9 Research questions and dissertation structure

There are many gaps in our knowledge of the dispersal behaviors and mating system of the terrapin. Furthermore, current data provide little information on the consequences of various human impacts on dispersal and mating behavior in the

terrapin. The overall aim of this study is to address the consequences of various human activities on the dispersal and mating behavior of the terrapin in order to provide information for conservation managers to maintain or enhance populations of diamondback terrapins. The major research questions addressed in this dissertation are as follows:

1) How does habitat fragmentation affect the dispersal of the diamondback terrapin?
2) What sex is primarily responsible for gene flow between localities?
3) How do the mating system and reproductive behaviors of the terrapin affect population-wide patterns of genetic diversity and gene flow and does sex ratio affect the mating system?

In Chapter Two, I used genetic datasets collected from six locations along the central coast of New Jersey, to explore the relationship between genetic distance and straight-line geographical distance as well as measures of effective geographical distance, using a landscape genetic approach. This chapter is currently in preparation for Plos Biology as:

> Sheridan CM, Scribner KT, Spotila JR, Bien WF, Avery HW. (*In prep*). Landscape Genetic Structure in a Highly Fragmented Ecosystem.

In Chapter Three, I used capture-mark-recapture and genetic datasets to investigate dispersal differences between males and females and to determine if nesting females are philopatric to natal nesting beaches. This chapter is currently in review in Molecular Ecology as:

> Sheridan CM, Spotila JR, Bien WF, Avery HW. (*In review*). Sex-biased dispersal, natal philopatry, and home range size of the diamondback terrapin, *Malaclemys terrapin. Molecular Ecology*.

In Chapter Four, I investigated the genetic mating system of four diamondback terrapin nesting beaches in Barnegat Bay and one nesting beach in the Chesapeake Bay. Specifically, I determined the frequency of clutches with multiple paternity at each nesting beach and assessed whether there were any direct initial reproductive advantages to multiple paternity. I also determined whether frequency of multiple paternity was correlated with population sex ratio and if females dispersed outside of their home ranges for mating. This chapter is currently in review in Molecular Ecology as:

> Sheridan CM, Spotila JR, Roosenburg WM, Bien WF, Avery HW. (*In review.)* Inter-population variation of multiple paternity in the diamondback terrapin (*Malaclemys terrapin*). *Molecular Ecology*.

In Chapter Five, I examined the relationship between female size and reproductive output. I also examined the latitudinal reproductive variation in the terrapin. Although, the analyses in chapter five were not specifically designed to investigate factors influencing genetic diversity in the terrapin, they revealed important insights into the reproductive ecology of the terrapin and insights about limits and variation in female reproductive output. Although multiple paternity can decrease coancestry among offspring, variation in reproductive output can increase the coancestry of offspring compared to populations in which there is no variance in female reproductive output (Scribner *et al.* 1993). Thus, populations with high

variance in reproductive output could be at higher risk for inbreeding. This chapter is currently in preparation for the journal Oikos as:

> Sheridan CM, Wnek JW, Spotila JR, Bien WF, Avery HW. (*In Prep*). Constraints on egg size, optimal egg size theory, and latitudinal reproductive variation in the diamondback terrapin (*Malaclemys terrapin).*

Lastly, Chapter Six provides a synthesis of the main findings of the dissertation, future directions for research, as well as broad implications for diamondback terrapin conservation. There is some unavoidable repetition in the introduction and methods sections of each chapter because this dissertation is written as a series of individual manuscripts. Each chapter includes an introduction with specific aims and hypotheses, as well as a discussion, which covers important aspects of the empirical data presented and places the results in the context of existing work and conservation implications.

CHAPTER 2: Landscape genetic structure of the diamondback terrapin (*Malaclemys* terrapin) in a highly fragmented ecosystem

2.1 Abstract

Habitat loss and fragmentation are the primary driving forces for loss of biological diversity worldwide. Habitat fragmentation can reduce effective dispersal of plant and animal populations and can affect species genetic diversity and long-term viability. Understanding how human altered landscapes affect gene flow between populations can provide conservation managers with valuable information to determine dispersal corridors. Coastal ecosystems are among the most highly fragmented ecosystems in the world and are continually threatened with further human development and future climate change. We used a landscape genetics approach to determine how habitat fragmentation due to human development in a highly impacted estuarine ecosystem affects gene flow in a model estuarine species, the diamondback terrapin (*Malaclemys terrapin*). Despite the low levels of spatial genetic structure ($F_{ST} = 0.002$) over a relatively large area (182 km^2), our landscape genetic approach was useful in identifying estuarine emergent wetland as a landscape feature necessary for effective dispersal of *M. terrapin.* Because historical losses of estuarine emergent wetland occurred from 1940-1970 and our model species is long-lived, we demonstrated that a landscape genetics approach can be used to determine the effects of landscape changes over ecologically relevant time scales. Our data also demonstrated that aquatic connectivity of habitats does not necessarily equate to genetic connectivity, even in an estuarine species such as the *M. terrapin.* The data

also suggested that direct measurements of dispersal in other semi-aquatic species may incorrectly attribute connectivity to the landscape features not ultimately necessary for gene flow.

2.2 Introduction

Habitat alteration represents the primary driving force for the loss of biological diversity worldwide (Vitousek *et al.* 1997). Anthropogenic habitat alteration and degradation has altered 39 to 50% of land on earth (Daily 1995; Vitousek *et al.* 1986). However, these values underestimate the impact of habitat transformation because it does not include the habitat that becomes fragmented when surrounding habitat has been altered (Vitousek *et al.* 1997). The effects of habitat fragmentation on animal populations have been documented extensively (Andren 1995; Fahrig 2003; Fahrig & Merriam 1994; Laurance & Bierregaard 1997; Prugha *et al.* 2008; Whitcomb *et al.* 1981; Wilcox & Murphy 1985). Habitat fragmentation can increase the rate of population extinction through genetic factors (Saccheri *et al.* 1998) and demographic factors (Boudjemadi *et al.* 1997). Effective dispersal (gene flow) affects probabilities of population viability and the ability of a population to adapt by reducing rates of loss of genetic diversity due to genetic drift, especially in small fragmented populations (Lynch & Lande 1998). Gene flow between populations is influenced by intrinsic factors such as innate dispersal ability and the breeding system of the species and extrinsic factors such as heterogeneity in landscape features or other environmental factors (Lowe *et al.* 2004; With *et al.*

1997). Understanding the effects of human activities on gene flow between animal populations can provide valuable information for conservation (Crandell *et al.* 2000; Crooks & Sanjayan 2006).

The discipline of landscape genetics i.e., the integration of the field of population genetics and landscape ecology, provides the ability to quantitatively determine the effects of landscape features, both natural and anthropogenic, on spatial genetic structure (Manel *et al.* 2003; Storfer *et al.* 2007). The incorporation of geographical information systems (GIS) and statistics into population genetics studies provides the ability to visualize and quantify the effects of natural and anthropogenic landscape features on spatial genetic structure in many species, including mammals, amphibians and reptiles (Arens *et al.* 2007; Blanchong *et al.* 2008; Clark *et al.* 2007; Emaresi *et al.* 2009; Funk *et al.* 2005; Piertney *et al.* 1998; Scribner *et al.* 2005; Spear *et al.* 2005; Stevens *et al.* 2006; Wang 2009). Several landscape genetics studies have shown that least-cost paths, incorporating landscape features with different costs, are better predictors of rates of gene flow than is straight-line (Euclidian) distance (e.g. Cushman *et al.* 2006; Lee-Yaw *et al.* 2009; Perez-Espona *et al.* 2008; Spear & Storfer 2008). The cost generally reflects some understanding of resistance or propensity to disperse through a landscape feature for a species (McRae 2006), but other factors such as thermal stress, predation risk, and energy expenditure can also contribute to the cost of traversing a landscape (Wang *et al.* 2009).

Coastal ecosystems are among the most productive ecosystems in the world, yet they are also among those at risk to multiple stressors including human

development and global climate change (Scavia *et al.* 2002). The United States coast, similar to other coasts around the world, has a long history of human development pressure (Walker 1990). While coastal zones only encompass 8% of the earth's land area, over 60% of the world's population and 53% of the U.S. population lives in a coastal zone (Culliton *et al.* 1990). Although development in coastal zones has been restrained by a variety of local, state, and federal laws, the pressure of human uses continues to grow (Bartlett *et al.* 2000). The northeast corridor of the United States (Washington, D.C. to Boston, MA) has the highest population density in the United States (Pulsipher & Pulsipher 2005). Within the northeast corridor, New Jersey has the highest population density, making the central coast of New Jersey an excellent location for studying the impacts of human development on highly altered coastal ecosystems.

One species that serves as an excellent example for addressing the effects of habitat fragmentation on coastal ecosystems is the diamondback terrapin, *Malaclemys terrapin*. The terrapin inhabits the coastal brackish estuaries and marshes along the Atlantic and Gulf coasts of the United States, from Corpus Christi, Texas to Wellfleet, Massachusetts (Iverson 1992). The diamondback terrapin is a habitat generalist that utilizes both the terrestrial and aquatic habitat of an estuary for foraging, mating, nesting and hibernation, thus, making it an excellent model organism for understanding change in estuaries comprised of both terrestrial and aquatic habitat.

Nesting ecology and population studies have documented that terrapins exhibit nest site fidelity (Auger 1989; Mitro 2003; Roosenburg 1996) and high fidelity to specific creeks (Gibbons *et al.* 2001) or sections of a river (Roosenburg *et al.* 1999). Male and female terrapins are typically recaptured within a few meters of their original capture location for up to three years (Lovich & Gibbons 1990), and similar results are seen after 16 years (Gibbons *et al.* 2001). Female terrapins often return to nest year after year in the same area (Burger 1977; Szerlag & McRobert 2007). Although strong data for site fidelity exists in demography studies, low to no genetic differentiation within eastern North Carolina (Hart 2005), southwestern Florida (Hart 2005), and South Carolina (Hauswaldt & Glenn 2005) suggests significant gene flow. Direct methods of measuring dispersal, such as mark-recapture or radio-tracking, may fail to detect long-distance or infrequent dispersal (Slatkin 1985) and only measure the ability of an organism to disperse, rather than the ability of an organism to disperse and successfully reproduce. Indirect methods of measuring of gene flow in the terrapin document sex-biased dispersal, with males as the primary disperser throughout the species range (Hart 2005; Chapter 3).

The Barnegat Bay Estuary is a 182-km^2 shallow lagoon-type estuary located along the central coast of New Jersey. Barnegat Bay is ecologically threatened by changes in water quality and quantity, habitat loss and alteration, fisheries declines, and other anthropogenic activities that have been ongoing since the first Europeans settled in the mid 17th century (BBNEP 2002). Currently, over 450,000 people live within the watershed, but land and water use doubles during the summer vacation

months (BBNEP 2002). In Barnegat Bay, 71% of the shoreline has been developed or altered by humans and has led to the loss of 28% of the historical estuarine emergent wetland (Lathrop *et al.* 1999). Most of this loss occurred from 1940-1970, prior to the Coastal Wetlands Law of 1970. From 1970-1995, an additional 1.5% was lost to development. The majority of estuarine wetland loss has occurred in the northern end of Barnegat Bay. The wetland loss is both spatially heterogeneous and has occurred over ecologically relevant times scales, especially for a turtle species that has a long generation time of approximately 10-40 years (generation time in closely related species: 10 years (*Chrysemys picta*; Wilbur 1975) to 37 years (*Emydoidea blandingii*; Congdon *et al.* 1993). In addition, over 63% of the developed shoreline is bulkheaded (Lathrop 1999), which could limit the ability of terrapins to move out of the water and onto land for nesting, foraging, or basking. Furthermore, road mortality is known to cause deaths of nesting females (Hoden & Able 2003; Szerlag & McRobert 2006).

Habitat alteration in Barnegat Bay estuary also occurs in the open water. The bay is dredged along the Intercoastal Waterway to maintain a depth of 2-4 m for navigation purposes. Boating traffic in the waterways is high from May-August (US Department of Homeland Security *et al.* 2009) and these months coincide with the active season of the diamondback terrapin (Yearicks *et al.* 1981). Diamondback terrapins are known to sustain injuries from boat propeller encounters (Gibbons *et al.* 2001; Tucker *et al.* 2001), but injury rates likely underestimate total boat encounters. Terrapins with major injuries from boat encounters have lower survivorship than

uninjured terrapins (Cecala *et al.* 2008), and thus major injuries could limit the ability of terrapins to disperse.

In this study, we used bi-parentally inherited microsatellite loci to assess the spatial genetic structure and the effects of landscape features on diamondback terrapin gene flow along the central coast of New Jersey. We used GIS techniques combined with population genetics and geographical statistics to assess the influence of several natural and anthropogenic landscape features on diamondback terrapin gene flow. We hypothesized that dispersal through some landscape features will involve higher costs, and therefore we expected that lower levels of gene flow would occur between areas separated by human impacted landscapes relative to natural areas. We expected that natural landscape features, such as areas with estuarine emergent wetland and open water (particularly shallow water), would promote diamondback terrapin gene flow and that anthropogenic landscape features, such as areas with deep water channels, roads, development, and bulkheading, would be a barrier to diamondback terrapin gene flow. Data will inform conservation decisions for diamondback terrapins by providing measures of population responses to current anthropogenic disturbance that may be extended spatially to other regions across the species' range, and temporally to predict the consequences of future events.

2.3 Methods

Study site and field sampling methods

In Barnegat Bay, the distribution of terrapins is heterogeneous and thus we could not sample individuals in a systematic grid-like manner (Epperson 1990). Individuals are found in habitat patches (estuarine emergent wetland surrounded by shallow water) that have been protected from development by local, state, and federal parks, refuges, and reserves. We chose six sampling locations based on the presence of nesting females, males, and juveniles within these protected habitat patches and our ability to capture at least 20 individuals at each location. However, sample sizes of 50-100 individuals are often considered minimal (Anderson *et al.* In Review) especially when gene flow is predicted to be high, so we sampled >100 individuals when possible. Based on mark-recapture, telemetry studies, and prior gene flow estimates of the terrapin, we expected that our sampling extent was large enough that the influence of gene flow from populations outside the study would not overwhelm the signature of landscape effects in the study area (*sensu* Anderson *et al.* In Review).

The Barnegat Bay Estuary study site (Figure 2-1) had several estuarine wetland areas (terrestrial habitat of Spartina sp., mud, and shallow water at high tide) that are protected from further development. These sites included Great Bay Boulevard Wildlife Management Area (GBWMA), the Edwin B. Forsythe National Wildlife Refuge (EBFNWR), Island Beach State Park (IBSP), and Cattus Island County Park (CI; from south to north). We divided samples from EBFNWR into North Forsythe and South Forsythe using Gunning River (300 m wide) as a boundary

because during the study period individuals captured were never captured on both sides of the river. In addition to blood samples collected within these four protected areas, we also collected samples from a site south of Barnegat Bay in Margate, NJ located within Brigantine Bay. The average straight-line distance between the mean center of sampling points was 35.45 km (minimum and maximum distances of 4.05 and 86.25 km, respectively).

We sampled terrapins between June-September in 2006-2008. We did not expect changes in genetic structure over this 3 year period because genetic structure is integrated over longer time scales, especially in this species with a >10 year generation time. We trapped terrapins using hoop nets, fyke nets, dip nets, and hand capture. We individually marked turtles by notching the marginal scutes of the carapace (Cagle 1939) and determined sex on the basis of carapace length, tail thickness, and cloacal positioning (Tucker *et al.* 2001). In total, we collected blood, tissue, or scute samples from 1558 individuals (482 males, 982 females, and 28 juveniles; see Figure 2-1 for sample size by location). We extracted blood samples via the subcarapacial sinus (Hernandez-Divers *et al.* 2002) or the brachial artery (Avery & Vitt 1984). We also collected samples of tail tissue or scutes from dead animals. We recorded GPS locations for each animal.

DNA laboratory procedures

We preserved blood samples for individual turtles on FTA® cards. Cards were stored at ambient temperature away from direct heat, moisture, and UV

exposure. We placed a sub-sample (1.2 mm disc (Harris Micro-Punch) from each card) in a PCR micro-centrifuge amplification tube. We rinsed samples once with 50 ul of 70% ethyl alcohol for 5 minutes, twice with 50 ul of FTA® purification reagent for 5 minutes, and twice with 50 ul of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) for 5 minutes. We dried sample discs for approximately 10-15 minutes on a heating block at 50^{o}C. We conducted PCR within 3 hours of disc washing.

We froze tissue or scute samples at -20^{o}C. We rinsed tissue samples twice with 200 ul of 1x Phosphate Buffer Saline Solution (1x, pH 7.4, 11.9mM Phosphates, 137mM Sodium Chloride, and 2.7 mM Potassium Chloride) prior to extraction. We extracted genomic DNA with the DNeasy Tissue Kit, following the manufacturer's instructions (QIAGEN).

We designed a 6-microsatellite loci protocol for polymerase chain reaction (PCR) and multiplexing. We selected loci from 16 microsatellite loci that were originally developed for the bog turtle (*Glyptemys muhlenbergii;* King & Julian 2004) and that successfully amplified and exhibited moderate to high levels of polymorphism in *Malaclemys terrapin.* Using allele frequencies of individuals sampled in Cape May, NJ and Sandy Hook, NJ (Hart 2005), we selected 6 microsatellite loci for this study based on levels of levels of polymorphism and heterozygosity. Selected primers were run in two multiplex PCR reactions (Plex 1: *Gmu*B08, *Gmu*D121, and *Gmu*D62; Plex 2: *Gmu*D87, *Gmu*D114, and *Gmu*D90). The forward primer was 5' modified with FAM, NED, or HEX fluorescent labels. Each 20 μL PCR reaction contained 5-15 ng of DNA or a 1.2 mm FTA blood punch, 0.3175

mM dNTPs, 1x GoTaq Flexi Buffer (Promega), 3.75 mM MgCl2, 0.2-0.25 μM of each primer, and 0.5 units of GoTaq polymerase (Promega). After PCR cycling (Hart 2005), we ran multiplex PCR products on an ABI 3100 capillary sequencer (Applied Biosystems) together with the internal size standard GENESCAN 500 ROX (Applied Biosystems). We conducted fragment analysis using the software Peak Scanner version 1.0 (Applied Biosystems).

Genetic diversity analyses

We used MICROCHECKER 2.2.3 (Oosterhout *et al.* 2004) to detect any genotyping errors, extreme stuttering, and null alleles. We calculated null alleles using a Monte Carlo simulation with bootstrapping to generate expected homozygote and heterozygote allele size difference frequencies which were then compared to the expected Hardy-Weinberg frequencies. We calculated genetic diversity measurements such as mean number of alleles per locus, allele frequencies, and gene diversity (Nei 1987) using FSTAT 2.9.3 (Goudet 2001). We used the program GenALex (Peakall & Smouse 2006) to calculated observed, H_O, and expected, H_E, heterozygosities. We tested for significant deviations from Hardy-Weinberg equilibrium and the presence of linkage disequilibrium using FSTAT 2.9.3 (Goudet 2001), with strict Bonferroni correction applied to adjust nominal alpha levels to account for multiple comparisons (Rice 1989).

Spatial genetic differentiation analyses

To estimate the extent of gene flow, we measured the level of genetic differentiation among *a priori* defined populations using traditional F-statistics (Wright 1978). We calculated F-statistics between pairs of populations using the multilocus estimator of F_{ST} (Weir & Cockerham 1984) implemented in GENEPOP 3.4 (Raymond & Rousset 1995). We obtained significance values over all loci using a Fisher's exact test in CHIFISH (Ryman 2006) which was estimated without bias using a Markov chain algorithm (Raymond & Rousset 1995) with 10,000 iterations. Microsatellites often have high allelic diversity and as a consequence the upper bound of F_{ST} is often <1 (Hedrick 2005). The G'_{ST} is a standardized measure of genetic distance based on Weir and Cockerham's (1984) adjustment of F_{ST} that divides the estimated F_{ST} by its upper limit. After we calculated the upper limit of F_{ST} for all population pairs (Meirmans 2006), we calculated G'_{ST} for all population pairs (Hedrick 2005).

Effects of landscape features on diamondback terrapin population structure

To test our null hypothesis that genetic differentiation was a function of straight-line or shoreline distance, we estimated the correlation coefficient between pairwise F_{ST} and straight-line geographical distance and between pairwise F_{ST} and shoreline distance with 1,000 bootstrap randomizations. To quantify the effects of several natural and anthropogenic landscape features on diamondback terrapin gene flow, we combined GIS techniques that quantified the matrix of available habitat

between sampling locales with measures of spatial genetic structure. We calculated the mean centers for each of the 6 sampling sites in ARCMAP version 9.3 (ESRI) using ARCMAP spatial statistic tools and geographical coordinates for each individual sampling location. We assessed the following landscape features for their influence on diamondback terrapin gene flow: open water, shallow water, deep water channels, development, estuarine emergent wetland (terrestrial habitat of *Spartina sp*., mud, and shallow water at high tide), roads, and bulkheading. We obtained digitized maps for each of these landscape features from various sources and processed the maps for our analysis as described below. We converted all maps to a 30 m grid cell resolution because further resolution significantly slowed our processor's computational ability.

We obtained road maps (TIGER) from the NJDEP Digital Data Downloads. The maps contain 2000 road information at the NJ state level and were in ArcView Shape file format. We converted the shapefiles for Atlantic (NJDEP *et al.* 2003a) and Ocean counties (NJDEP *et al.* 2003b) to raster format in 30 m resolution and we added the rasters together to form a single map layer.

We obtained the 2001 Land Cover Classification Map of New Jersey in Arc/Info raster grid format (CRSSA 2004a). The 30 m resolution map was classified by CRSSA from satellite imagery processed by the U.S. Geological Survey EROS Data Center and contains 11-class land cover types, including open water, development (>25% impervious surface), estuarine emergent wetland, and other land types.

We obtained a bottom type map in Arc/Info raster grid format and 1 m resolution based on imagery from 2003 (CRSSA 2004b). The map contains 8-class types, including shallow water (<1.5-2.0m) and deep water channels (>1.5-2.0m). We converted the maps to 30 m resolution.

The digital line graphs of bulkheading were originally derived by CRSSA from a 1: 24,000 scale USGS hydrography digital line graph data set (CRSSA 1999). To assess the influence of bulkheading on gene flow, we converted the digital line graph to a raster data set with 30 m resolution and we reclassified the individual bulkheads into one bulkhead class. Both the bottom type and bulkheading maps did not include spatial data south of the Great Bay sampling location and as a result spatial analyses using features obtained from these maps excluded the Margate, NJ sampling location.

Dispersal models were created using a least-cost path analysis. Because there is a lack of information as to which landscape features facilitate or act as barriers to diamondback terrapin dispersal and of the relative costs for diamondback terrapins to cross different landscape features, we used a relatively new method (Perez-Espona *et al.* 2008) to assess the effects of each landscape feature on diamondback terrapin gene flow. Each of the 7 landscape features were analyzed separately in a least-cost path analysis. Using spatial analyst in ARCMAP (ESRI), we created a cost raster by considering each map as a grid and assigning to each cell (30 X 30 m) on the grid a cost. We assigned all cells a cost = 1, except those containing the landscape feature of interest. We gave the feature of interest a range of 18 arbitrary cell cost values

(0.00003, 0.0001, 0.0003, 0.001, 0.003, 0.01, 0.03, 0.1, 0.3, 3, 10, 30, 100, 300, 1,000, 3,000, 10,000, and 30,000). We choose the values so that the logarithm of the cost values increased in uniform steps. We then created a cost weighted distance raster for each sampling site. In the raster we assigned each cell a value that is the least accumulative cost of moving to the mean center of the sampling site. We calculated the least cost path between each of the sampling sites using the cost weighted distance raster. We recorded the total accumulative cost of the path, i.e. *sensu stricto* the least-cost distance, in a matrix for each combination of landscape feature/arbitrary cost value.

Using the resulting 126 matrices, we determined which of the landscape features/arbitrary cost value combinations better described diamondback terrapin gene flow. We performed correlation analyses between the population pairwise F_{ST} and each of the resulting 126 (7 features X 18 cost values) least-cost distance matrices. We obtained p-values from correlation analyses after 1,000 bootstrap permutations. We also obtained p-values from Mantel tests with 1,000 permutations (results were similar to correlation analyses and are not provided). For each of the 7 landscape features, we produced a set of 18 correlation coefficients. The values represent the correlation between the measured genetic differentiation and each of the 18 cost functions of the landscape feature. We assumed that the cost value with the highest correlation coefficient was the one that maximized the relationship between genetic differentiation and the landscape feature of interest. When the cost value for the feature of interest was <1 and there was a positive correlation between the genetic

differentiation and least-cost distance, we assumed that the feature of interest facilitated gene flow. When the cost value for the feature of interest was >1 and there was a positive correlation between genetic differentiation and least-cost distance, we assumed that the feature of interest was a barrier to gene flow.

To determine whether the models significantly explained the genetic variation better than the straight-line distance or shoreline distance models, we used a parsimonious framework for model comparison that aimed to rank the candidate models (Burnham & Anderson 2002) in order to select the most likely model of landscape influence on genetic structure in the terrapin. Our approach was based on the calculated value of Akaike's information criterion (AIC; Akaike 1974) as a measure of the fit of the model (Burnham & Anderson 2002). Because our sample size was low, we calculated AIC_c, which includes a bias adjustment for small sample sizes (Hurvich & Tsai 1989). We calculated the probability that any given model was the best fit out of those tested using the Akaike weight of each model (wAIC; Anderson *et al.* 2001). We used a cut-off point for model selection of Akaike weights that were within 10% of the model with the highest weight (Royall 1997).

For the model that best fit the data, we calculated the total distance traversed through each landscape type for each least-cost path in the model. For example, in the land cover map we determined the proportion of the least-cost path that crossed open water, development, forest, grassland and other landscape types when the model maximized movement through estuarine emergent wetland. To calculate the proportion, we converted the least-cost path to a raster with 10 m resolution and

reclassified all cells within the line to a value of 1. We added the least-cost path raster to the landscape raster, in which each landscape type had a different value. We recorded the attribute table from the resulting raster. We calculated the proportion of each landscape feature that accounted for the feature not tested in the model (in this example estuarine emergent wetland), to determine which features contributed the most to the increased cost-distance between sampling sites.

Finally, using the best-fit model (based on AIC) that described the single landscape feature shaping gene flow in the terrapin (e.g. estuarine emergent wetland), we assessed several least-cost models incorporating the single landscape feature shaping gene flow as well as the predominate landscape features that contributed to the increased cost-distance between sampling sites. In these mixed landscape feature models, we used the same cost value for the single landscape feature as in the best-fit single landscape feature model, but varied the cost of the predominate landscape features that contributed to the increased cost-distance between sampling sites. With these mixed models, we attempted to refine our best-fit single landscape feature model with a model incorporating a realistic approach that several landscape features, each with relatively different costs, likely influence terrapin gene flow. We assumed that the mixed model with the highest correlation coefficient was the one that maximized the relationship between genetic differentiation and the multiple landscape features of interest. We assessed whether the mixed model with the highest correlation coefficient had better overall support than the single landscape feature models, using AIC (Akaike 1974).

2.4 Results

Genetic diversity analyses

The number of alleles per locus ranged from 10 (*Gmu*D114 and *Gmu*D90) to 17 (*Gmu*D121), with a mean of 12.33 alleles per locus. Mean expected heterozygosity (H_E) and observed heterozygosity (H_O) per locus across the study area were 0.808 (range: 0.677-0.876) and 0.825 (range: 0.696- 0.875), respectively. Allelic richness per locus (scaled to 22 individuals) ranged from 6.8 (*Gmu*D114) to 11.5 (*Gmu*D121). Gene diversity per locus ranged from 0.727 (*Gmu*D114) to 0.891 (*Gmu*D121). Mean number of alleles per locus at each sampling location was 10.36 (SE 0.57, range: 8-12). Mean H_O of sampling locations was 0.82 (SE 0.009, range 0.780-0.843). Mean allelic richness of sampling locations was 8.44 (SE 0.09, range 8.0-8.65). Null alleles were not detected for any locus (a = 0.05). No departures from Hardy-Weinberg equilibrium were detected within any of the sampling sites at any loci, within any of the sampling sites over all loci, or over all loci and all sampling sites. No linkage disequilibrium was detected for any pair of loci at any sampling site after Bonferroni correction (a = 0.05, k = 90, $p < 0.001$; Rice 1989).

Population differentiation analyses

Population differentiation across the whole study area was low ($F_{ST} = 0.002$), but significantly different from zero (Fisher exact test, $p < 0.001$). Pairwise F_{ST} values ranged from 0 (GB-MAR) to a maximum value of 0.0074 between CAT-IBSP (Table 2-1). Using Fisher's exact tests, the number of significant pairwise comparisons was

8 out of 15 (Table 2-1). High heterozygosity reduced the maximal F_{ST} value to far less than 1 (maximum range: 0.180-0.195). Standardizing these values yielded a G'_{ST} of 0.011 across the whole study area and a range of 0 to 0.038 for pairwise comparisons (Table 2-1). G'_{ST} and F_{ST} were highly correlated ($r = 0.99$, $p < 0.001$).

Effects of landscape features on diamondback terrapin population structure

The correlation between straight-line geographical distance and genetic differentiation (F_{ST}) was not significant ($r = 0.18$, $p = 0.26$; Table 2-2). The correlation between shoreline distance and genetic differentiation (F_{ST}) was not significant ($r = 0.12$, $p = 0.30$; Table 2-2).

We chose to use F_{ST} values for the correlation between the genetic distances and landscape least cost-distances (Table 2-1), but since F_{ST} and G'_{ST} were highly correlated, similar correlation coefficients and trends were obtained with G'_{ST} (not shown). Several features did not have significant correlation coefficients for any of the cost values: open water, development, roads, deep water channels, and bulkheading (Table 2-2). The optimal cell cost for estuarine emergent wetland was 0.003 and it had a significant positive correlation ($r = 0.652$, $p = 0.006$) between least-cost distance and genetic differentiation (Figure 2-2). Since the optimal cell cost was <1 and the correlation was positive, estuarine emergent wetland can be identified as a landscape feature that facilitated diamondback terrapin gene flow. The optimal cell cost for shallow water was 1,000, in which there was a marginally non-significant negative correlation ($r = -0.551$, $p = 0.08$). Although the optimal cell cost was >1, the

relationship was negative. Therefore shallow water was identified as a landscape feature that may facilitate diamondback terrapin gene flow.

The estuarine emergent wetland model in which estuarine emergent wetland was given a cost value of 0.003, exceeded all other models (Table 2-2). This model had the highest significant regression coefficient (0.652) and the wAIC values excluded all other single landscape models as candidate models (Table 2-3).

In the estuarine emergent wetland model where estuarine emergent wetland had a cell cost of 0.003, the average proportion of each least-cost path in the model that passed through cells with a cost of 1 (i.e. not estuarine emergent wetland) was 0.16 (0.10-0.23, 95% confidence interval). Open water and development accounted for the majority of cells with a cost of 1 in the least-cost paths (0.46 [0.33-0.58; 95% confidence interval] and 0.33 [0.19-0.46; 95% confidence interval], respectively). This indicated that when estuarine emergent wetland has a cell cost of 0.003 and all other landscape types have a cost of 1, open water and development were the primary contributors of increasing cost.

Our mixed model analysis included estuarine emergent wetland with a cell cost of 0.003, development with a cell cost ranging from 1 to 0.2, and open water with a cell cost ranging from 1 to 0.2. Among the mixed models, we found that the model in which development was given a cell cost of 0.5 and open water was given a cell cost of 1 (MIXED6) was the model with the highest correlation coefficient ($r = 0.672$; Table 2-4). While the AIC for MIXED6 (Figure 2-3) was lower (-192.2) than the AIC for the single landscape feature model of estuarine emergent wetland (-

190.8), both models wAIC values were within 10% of one another and thus they were both supported.

2.5 Discussion

Diamondback terrapins spend their active season primarily in aquatic habitats and similar to other aquatic turtles are thought to leave the water only to bask, nest, or occasionally migrate to a new aquatic habitat (Stephens & Wiens 2003). Few studies of aquatic turtles have used a landscape genetics approach to assess the impact of landscape features on gene flow and there are no studies implementing least-cost distances or resistance surfaces in aquatic turtles (Alacs *et al.* 2007). In some population genetic studies of aquatic turtles, higher rates of gene flow occur between contiguous or aquatically connected lake and riverine habitats than aquatic habitats separated by upland terrestrial habitat (*Trachemys scripta*; Scribner *et al.* 1986). Gene flow in other aquatic turtle species indicates that genetic structuring occurs according to the hierarchical system of rivers and streams within drainages and that limited gene flow occurs between different drainages (*H. maximiliani;* Souza *et al.* 2002, *Podocnemis unifilis*: Escalona *et al.* 2009, *Macrochelys temminckii*; Echelle *et al.* 2009). Thus, data for many aquatic turtle species suggest that effective dispersal occurs primarily via aquatic habitats. Additional studies indicate that gene flow is primarily a function of distance rather than watershed (*Emydoidea blandingi;* Mockford *et al.* 2005) or that dispersal occurs both aquatically and terrestrially (*Glyptemys insculpta;* Castellano *et al.* 2009). Our study is novel because it is the first

fine-scale study of an aquatic or semi-aquatic turtle that demonstrates that gene flow occurs primarily in estuarine emergent wetland (terrestrial habitat of *Spartina sp*., mud, and shallow water at high tide). Maximum tidal range on spring tides in Barnegat Bay was approximately 0.16 m. Thus, the majority of estuarine emergent wetland was not submerged during high tides.

In our study, straight-line geographical distance between populations did not explain the spatial genetic structure of terrapins across the study area, despite the fact that all populations are aquatically connected. Furthermore, contrary to our expectation that terrapins dispersed primarily in open water, our individual landscape models did not identify open water as a landscape feature that facilitated gene flow (Table 2-2). This suggested that terrapins did not utilize open water for effective dispersal. Even when accounting for the depth of the water to include the possibility that boat traffic could alter terrapin dispersal ability, neither deep water nor shallow water channels significantly explained the spatial genetic structure (Table 2-2). Although, our mixed model analysis (Table 2-4) indicated that open water was one of the primary landscape features that reduced gene flow. The landscape genetic data are supported by radio tracking data of terrapins. Diamondback terrapins tend to spend more time on the salt marsh rather than in open water of creek channels in South Carolina (Harden *et al.* 2007) and terrapins in a North Carolina spent more time in low marsh habitat than in other available habitat (Spivey 1998)

Hatchling and juvenile terrapins utilize terrestrial habitats as a strategy to deal with high osmotic stress in the high salinity environments (Kinneary 2008). However,

shoreline geographical distances between populations did not explain the spatial genetic structure of terrapins across the study area. Shoreline can consist of many landscape types (e.g. forest, unconsolidated shoreline, emergent wetland, and developed land). Thus shoreline may not have explained the spatial genetic structure because it did not accurately describe the landscape types necessary for terrapin physiology. Terrapins rely on estuarine emergent wetland for many biological functions including: thermoregulation, osmoregulation, and foraging. Terrapins thermoregulate on mud flats at the edges of wetlands (personal observation). Radio telemetry combined with temperature profiles of terrapins documents that they thermoregulate on emergent wetland comprising of *Spartina* grasses (Harden *et al.* 2007). Terrapins utilize the buildup of fresh water from rainfall in wetland pools or on *Spartina* grasses for behavioral osmoregulation (Davenport & Macedo 1990). Terrapins also feed on marsh snails (*Littorina irrorata)*, fiddler crabs (*Uca pugnax*), marsh crabs (*Sesarma reticulatum*), marsh clams (*Polynesoda caroliniana*), small barnacles *(Balanus)*, and blue crabs (*Callinectes sapidus*; Tucker *et al.* 1995), many of which are found only on the marsh surface or at the tidal interface. Indeed, estuarine emergent wetland landscape models (both in the single landscape feature model and the mixed models) predicted that the degree of wetland connectivity between populations significantly explained the variation in spatial genetic structure better than any other model (Tables 2-2 and 2-3). Therefore, we identified estuarine emergent wetland as the primary landscape feature that facilitated gene flow in *M. terrapin.* Indirect estimates of gene flow predict that males are the individuals

primarily responsible for gene flow (Hart 2005; Chapter 3), thus we can conclude that estuarine emergent wetland is necessary to maintain gene flow via males in the diamondback terrapin. We expect that across this species' range estuarine emergent wetland will remain a feature that facilitates dispersal.

Our study clearly identifies the importance of estuarine emergent wetland as a landscape feature that maintains gene flow in the diamondback terrapin and points to the need to conserve this natural feature to maintain connectivity between populations. Furthermore, findings from this investigation highlights that aquatic connectivity does not equate to genetic connectivity between populations in euryhaline species, such as the diamondback terrapin. This contradicts conventional wisdom; and such misconceptions could undermine efforts to maintain gene flow and genetic diversity of populations. Landscape genetic studies should be used to determine the relative importance of estuarine emergent wetland for dispersal in other semi-aquatic estuarine species such as saltmarsh water snakes (*Nerodia clarkia*), fiddler crabs (*Uca spp.*), marsh crabs (*Sesarma reticulatum)*, saltmarsh pulmonate gastropods (*Melampus* and *Ovatella spp.*), muskrats (*Ondatra zibethicus)*, saltmarsh periwinkles (*Littoraria irrorata*), and a variety of euryhaline plant species (such as *Spartina spp.* and *Salicornia spp.*).

Several landscape features, such as roads and bulkheading, failed to explain the variation in spatial genetic structure in our study. Roads (or bulkheading) are primarily factors encountered by females during the nesting season when females search for a suitable nesting area (Szerlag & McRobert 2006). It is possible that these

features do not affect gene flow because females may not be responsible for gene flow in the terrapin. Alternatively, males and juveniles occasionally encounter roads and can be affected by vehicle strikes (Hoden & Able 2003). Many roads (or bulkheading) that traverse or block access to estuarine wetland are relatively new features (e.g. Great Bay Boulevard circa 1990; Avissar 2006), compared to estuarine wetland loss (circa 1940- 1970; Lathrop 1999). Lag time in spatial genetic structuring in response to reduced gene flow (Anderson *et al. In Review*) could result in the inability of our landscape models to explain gene flow responses to relatively newer landscape features. Because spatial genetic structure is affected by both gene flow and genetic drift, large population sizes could result in longer lag times (Anderson *et al. In Review*). Furthermore, roads do not always have high levels of traffic and some individuals may disperse across the roads during these times. Bulkheading may contain breaks that are large enough for terrapins to occasionally disperse around them. While our data suggest that these features are not responsible for the current measured level of gene flow in the terrapin, these features may affect the measured levels of gene flow in the future.

Currently the objectives of most landscape genetic studies, including this study, are to identify landscape features that influence genetic connectivity, but landscape genetics studies can also be developed to predict the impacts of future environmental change on connectivity for a species (Spear *et al. In Review*). For example, sea level rise, due to global climate change, is projected to cause major losses of estuarine emergent wetland, especially in areas where shoreline

development will further impinge the landward migration process (Cooper *et al.* 2005; Kana *et al.* 1988). In Barnegat Bay, approximately 29% of potential tidal marsh retreat area is limited by developed features and roads (Lathrop Jr. & Love 2007). Limited retreat areas occur in the northern portion of the bay and along the backsides of the barrier islands, whereas southern Barnegat Bay has comparatively more unrestricted retreat zones due to federal and state wildlife refuges protecting coastal wetlands and uplands (Lathrop Jr. & Love 2007). Habitat models in relation to predicted sea level rise in Barnegat Bay have been developed (Strange *et al.* 2008). Our future studies will involve utilization of these sea level rise models to determine specific areas where sea level rise will threaten future population connectivity of the diamondback terrapin. It is clear from this study that there is a need to conserve tidal marsh retreat areas by limiting new development that would impede the landward migration process of tidal wetlands in order to prevent further reductions in gene flow in *M. terrapin* in Barnegat Bay as well as along the entire species range along the Atlantic and Gulf coasts.

The spatial genetic structure of *M. terrapin* was generally low (F_{ST} = 0.002, G'_{ST} = 0.011), over a relatively large area (182 km^2). The degree of genetic differentiation found in this study was comparable to that found for the diamondback terrapin in other study sites (South Florida, F_{ST} = 0.006; North Carolina 0.005; Hart 2005) and (South Carolina, F_{ST} = 0.001; Hauswaldt & Glenn 2005). Even with correction for high allelic diversity, genetic differentiation (G'_{ST}) in this study indicated little genetic differentiation (< 0.05; Wright 1978). Because genetic

differentiation is affected by both gene flow and genetic drift, low levels of genetic differentiation can be due to several factors other than high levels of gene flow, especially in iteroparous species with large effective population sizes, overlapping populations, long generation times, and multiple paternity. Despite low levels of genetic differentiation, our landscape genetic approach was useful in identifying estuarine emergent wetland as an important landscape feature necessary for effective dispersal in *M. terrapin* and thus demonstrated the power of a landscape genetics approach to determine how anthropogenic landscape changes are affecting species over ecologically relevant time scales (max. of 7 generations in *M. terrapin*).

Typically, management plans of aquatic organisms, such as frogs, salamanders and turtles include a buffer around individual wetlands because these organisms migrate annually between aquatic and terrestrial habitats to forage, reproduce, and overwinter (Semlitsch & Bodie 2003). These organisms are generally thought to be philopatric to individual wetlands and thus recommended buffer zones generally only include core habitats (Semlitsch & Bodie 2003), but ultimately conservation and management plans must consider both local and landscape dynamics (Semlitsch 2000). Since juveniles and males are the primary dispersers in amphibians and reptiles (Berven & Grudzien. 1990; Breden 1987; Gill 1978; Morreale *et al.* 1984; Parker 1984), management plans need to consider how landscape features affect dispersal in these individuals. Management plans based on movements measured in individuals not responsible for gene flow may fail to protect the habitat needed to maintain gene flow and could lead to reduced genetic diversity.

Our results reveal that the methods implemented in this study can be used to determine habitat features needed for effective gene flow and these methods can be utilized regardless of the knowledge of the stage or sex that disperses.

Table 2-1 Genetic and geographical distances between the six diamondback terrapin populations sampled in Barnegat Bay, NJ. Genetic distance (upper diagonal) is represented by F_{ST} (and G'_{ST}) and geographical distance in km (lower diagonal). Significant genetic differentiation is indicated by an asterisk (Fisher's exact test $p < 0.05$).

Population	CAT	IBSP	NF	SF	GB	MAR
CAT		0.0074 (0.038)*	0.0051 (0.027)	0.002 (0.011)	0.0042 (0.022)	0.0062 (0.032)*
IBSP	21.42		0.0013 (0.007)*	0.0049 (0.027)*	0.0027 (0.015)*	0.0023 (0.012)*
NF	29.70	9.55		0.0021 (0.012)*	0.0012 (0.007)	0.0028 (0.015)*
SF	33.77	13.62	4.05		0.0018 (0.010)	0.0027 (0.015)
GB	56.92	36.78	27.20	23.13		0 (0)
MAR	86.25	66.11	56.53	52.46	29.31	

*Population acronyms, locations, and sampling sizes are described in Fig. 2-1.

Table 2-2 Correlation coefficients between genetic differentiation and the least cost paths generated for the range of cell cost values assessed. Correlation coefficients with significant p-values (<0.05) after bootstrapping (N = 1000) are shown in bold. The 'optimal' cell cost value chosen for each of the landscape features with significant p-values is shown with an *. The analysis values highlighted in gray added significantly to the null models (straight line distance and shoreline distance) based on AICc. Data are presented for 12 of the 18 tested cost values, but results are similar with the full dataset.

	Cost values											
	0.001	0.003	0.01	0.03	0.1	0.3	3	10	30	100	300	1000
Open Water	-0.237	-0.202	-0.159	0.006	0.091	0.135	0.160	0.137	0.093	0.123	0.074	0.043
Development	-0.152	-0.084	-0.092	-0.093	0.078	0.145	0.143	0.153	0.102	-0.018	-0.018	-0.060
Emergent Wetland	**0.645**	**0.652***	**0.636**	**0.610**	**0.520**	0.419	0.121	0.108	0.000	-0.148	-0.211	-0.257
Roads	-0.197	-0.035	-0.267	-0.180	0.140	0.013	0.145	0.138	0.144	0.133	0.089	-0.111
Shallow Water	-0.030	-0.043	-0.049	-0.031	0.028	0.115	-0.044	-0.097	-0.131	-0.239	-0.380	-0.551
Deep Water Channels	-0.397	-0.373	-0.405	-0.469	-0.160	-0.052	0.088	0.113	0.123	0.090	0.067	0.104
Bulkheading	0.170	0.144	0.133	0.141	0.054	0.062	-0.028	-0.017	0.006	-0.037	-0.073	-0.031
Straight Line Distance	0.182											
Shoreline Distance	0.120											

Table 2-3 AIC_c, ΔAIC, and wAIC values for each least-cost model in Barnegat Bay, NJ. For each landscape type, AICc values were calculated based on the individual landscape feature model with the highest correlation coefficient (r).

Model	AIC_c	ΔAIC	wAIC
MIXED6	-192.22	0.00	0.64
Emergent wetland	-190.8	1.39	0.32
Roads	-183.9	8.33	0.01
Euchudian distance	-183.6	8.60	0.01
Open Water	-183.6	8.60	0.01
Development	-183.3	8.87	0.01
Shoreline	-183.1	9.14	0.01
Shallow water	-124.9	67.35	0.00
Deep Water	-122.4	69.82	0.00
Bulkheading	-121.2	71.00	0.00

Table 2-4 Correlation coefficients between genetic differentiation and the least cost paths of seven mixed models. The cell cost values for each landscape feature in the model are given. Correlation coefficients with significant p-values (<0.05) after bootstrapping (N = 1000) are shown in bold. The 'optimal' mixed model with the highest correlation coefficient is shown with an *.

Model	Water Cell Cost	Development Cell Cost	Estuarine Emergent Wetland Cell Cost	Correlation coefficient (r)	p-value
MIXED1	0.2	1	0.003	0.488	0.109
MIXED2	0.5	1	0.003	**0.590**	0.037
MIXED3	0.8	1	0.003	**0.643**	0.004
MIXED4	1	1	0.003	**0.647**	0.013
MIXED5	1	0.8	0.003	**0.665**	0.006
MIXED6	1	0.5	0.003	**0.672***	0.001
MIXED7	1	0.2	0.003	**0.622**	0.011

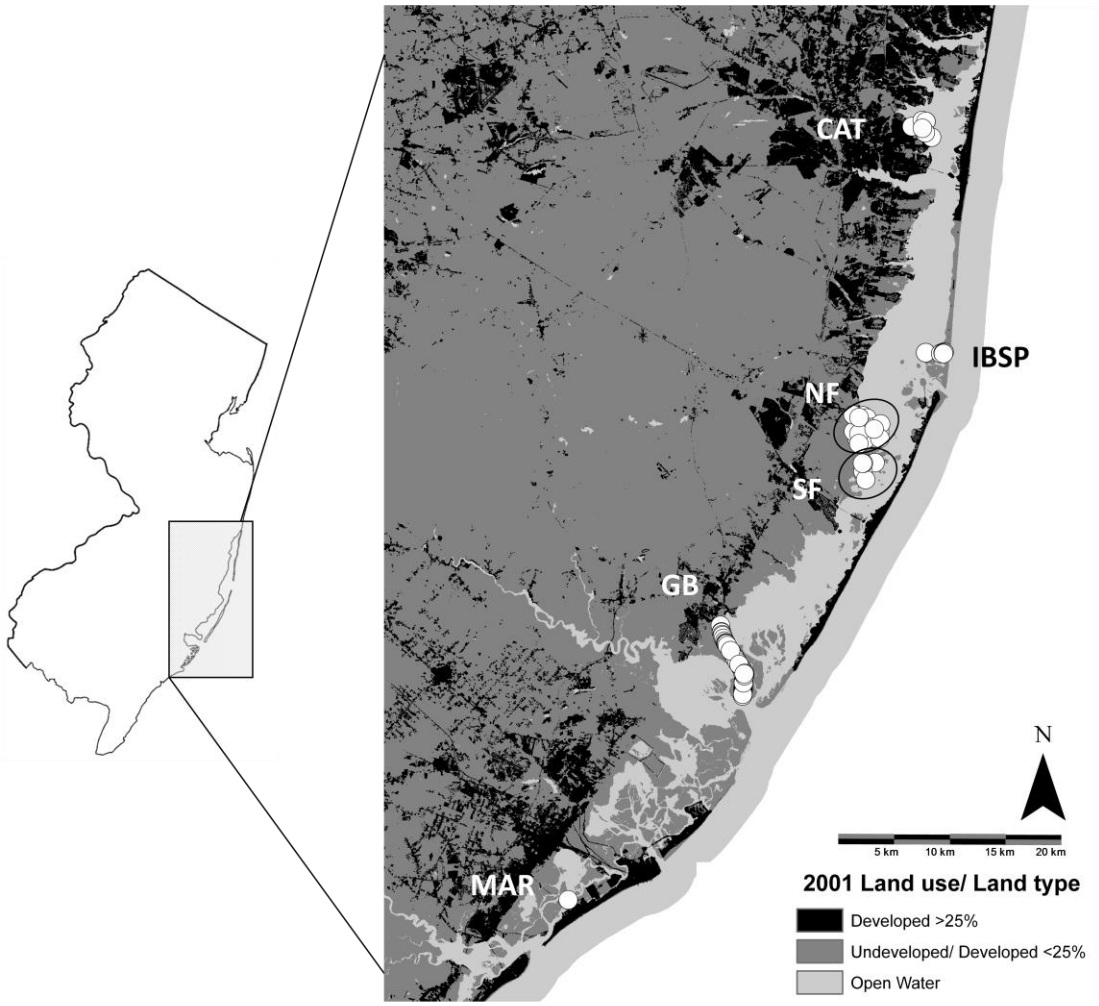


Fig 2-1. Map of sampling sites on a 2001 land use map. White circles indicate sampling areas at each location, while the two black circles discriminate between North and South Forsythe. Inset indicates general location of study site on a map of New Jersey. Sample sizes of diamondback terrapins are as follows North Forsythe (NF; n=1000), South Forsythe (SF; n=120), Great Bay (GB; n=101), Island Beach State Park (IBSP; n=259), Cattus Island County Park (CAT; n=22), and Margate (MAR; n=56).

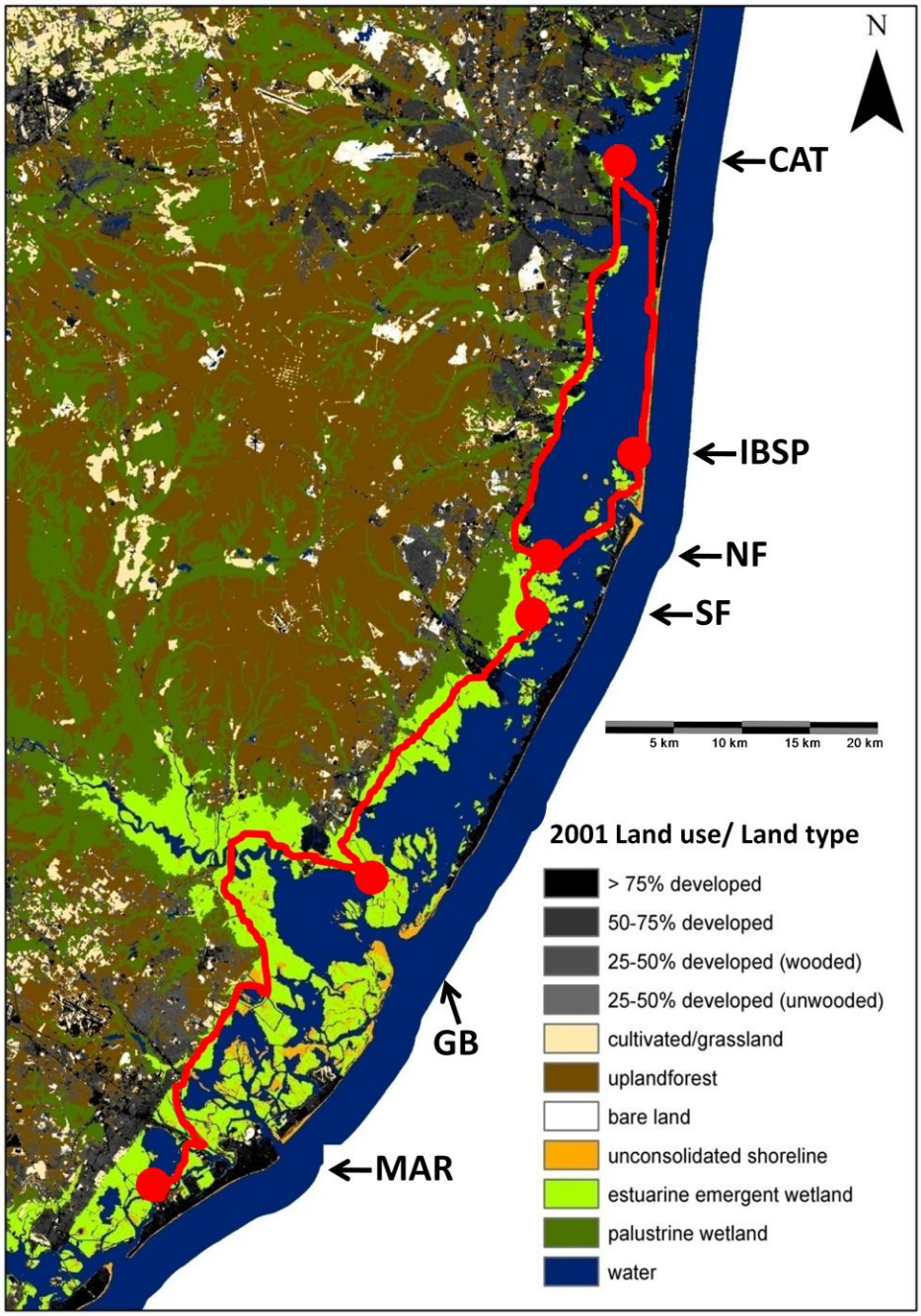


Fig. 2-2. Representative least-cost paths (in red) between sampling locations in Barnegat Bay, NJ when estuarine emergent wetland has a cost of 0.003 per 30 m^2 and all other landscape types have a cost of 1 per 30 m^2. This model explains 42.5 % of the genetic differentiation between diamondback terrapin sampling locations.

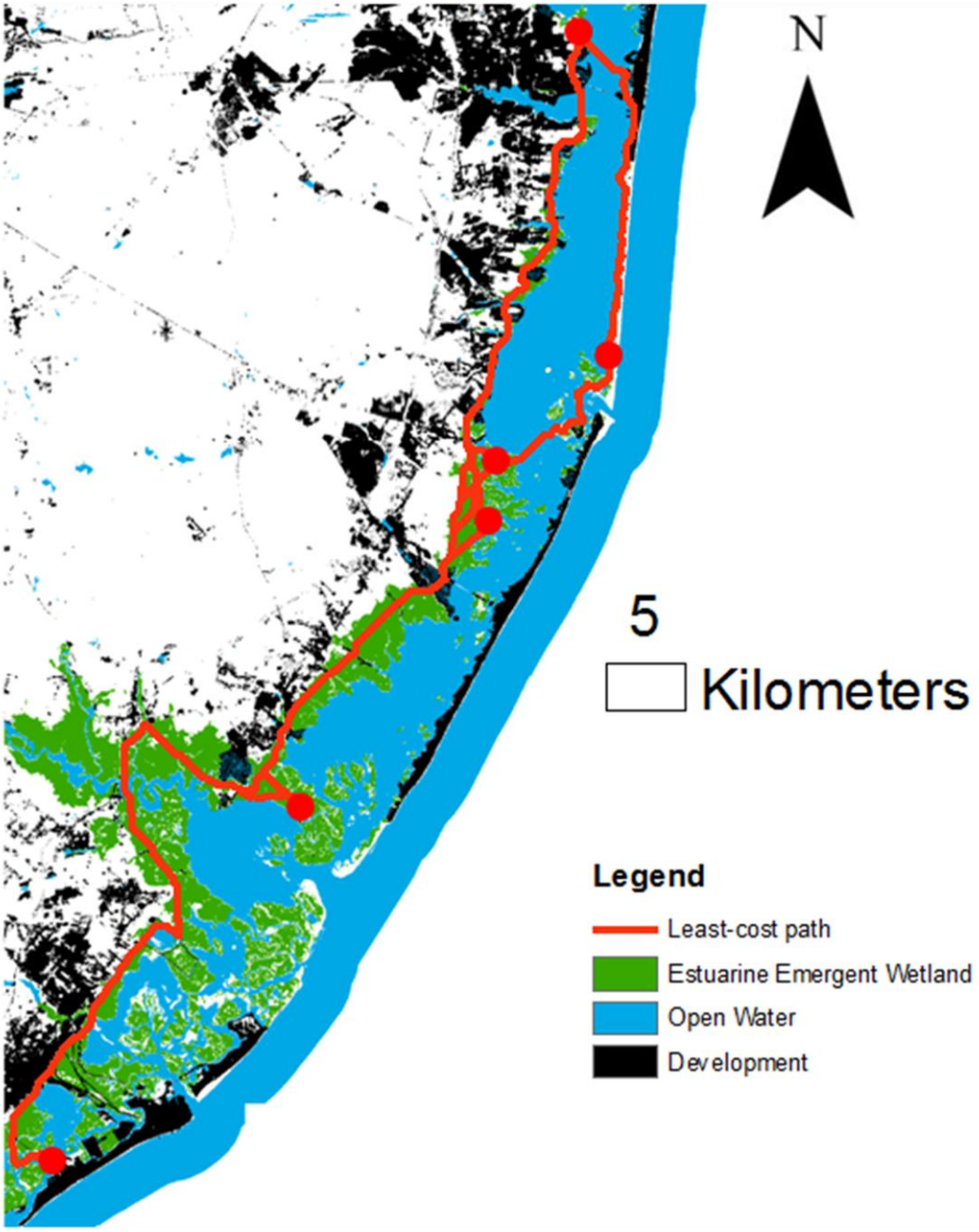


Fig. 2-3. Representative least-cost paths (in red) between sampling locations in Barnegat Bay, NJ when estuarine emergent wetland has a cost of 0.003 per 30 m^2 , open water has a cost of 1 per 30 m^2 and development has a cost of 0.5 per 30 m^2.This model (MIXED6) explains 45.1 % of the genetic differentiation between diamondback terrapin sampling locations.

CHAPTER 3: Sex-biased dispersal, natal philopatry, and home range movements of the diamondback terrapin, *Malaclemys terrapin*

3.1 Abstract

Nesting ecology and population studies indicate that diamondback terrapins (*Malaclemys terrapin*) exhibit nest site and habitat fidelity. However, genetic studies indicate high levels of gene flow. Because dispersal affects the genetics and population dynamics of a species, we used six highly polymorphic microsatellite markers to investigate sex-biased dispersal and natal philopatry of *M. terrapin* in Barnegat Bay, NJ. We compared results of spatial autocorrelation analysis, assignment methods, and Wright's F_{ST} estimators to movements measured via capture-mark-recapture analysis. Capture-mark-recapture analysis indicated that most individuals have relatively small home ranges (<2 km), with mature females having greater movements within home ranges than males. Mean assignment indices and first generation migrant tests indicated that mature males were more prone to disperse than mature females, but *per capita* there are more female than male dispersers. Thus the relative importance of males and females on gene flow in this species may change with population sex ratios. Spatial autocorrelation analysis indicated that mature females exhibited natal philopatry to nesting beaches, but first generation migrant tests indicated that some females failed to nest on natal beaches. Finally, we discuss the important conservation implications of male-biased dispersal and natal philopatry in the diamondback terrapin.

3.2 Introduction

Dispersal affects the genetics and population dynamics of a species. One important type of dispersal pattern is sex-biased dispersal, in which one sex is philopatric while the other sex disperses away from its natal area (Pusey 1987). Sex-biased dispersal can be measured using either direct or indirect methods (Slatkin 1985). Direct methods, such as mark-recapture or radio-tracking, have been used for many years and have been useful in identifying the movements of many vertebrates. However, they may fail to detect long-distance or infrequent dispersal (Slatkin 1985) and frequently measure movements within home ranges rather than true dispersal. Direct measurements of disperal also do not always reflect the movement of genes. Dispersal and gene flow are only synonomous if the migrant reproduces effectively in the location to where it dispersed (Whitlock & McCauley 1999).

Indirect methods are primarily based on genetic differences in populations and can provide a more complete analysis of sex-biased dispersal. Indirect estimates of effective dispersal (reviewed in Goudet *et al.* 2002; Manel *et al.* 2005; Prugnolle & de Meeus 2002; Slatkin 1985) include measures of genetic differentiation using bi-parentally inherited markers and uniparentally inherited markers. Differences in levels of genetic differentiation as measured by genetic markers with different modes of inheritance, such as sex-specific and autosomal nuclear markers, can indicate sex-biased dispersal (Prugnolle & de Meeus 2002). Conclusions based on this method need to be interpreted carefully because differences in mutation rates (Balloux *et al.* 2000) and effective population sizes (Chesser & Baker 1996), can lead to differences

in the amount of genetic differentiation, rather than differences in dispersal of the sexes.

Indirect methods that use bi-parentally inherited markers include assignment methods, relatedness with respect to geographic distance (e.g., Mantel tests or spatial autocorrelation), and comparison of Wright's F_{ST} estimators computed both for males and females. The F-statistic estimates (such as F_{ST}) mostly reflect historical dispersal behavior (Bossart & Prowell 1998), while individual-based statistical techniques such as assignment indices and spatial autocorrelation analyses are more useful for detecting current dispersal behavior (Double *et al.* 2005; Paetkau *et al.* 2004; Peakall *et al.* 2003).

Positive local spatial genetic structure, in which relatedness between individuals declines with increasing geographical distance, results from restricted dispersal within populations (Peakall *et al.* 2003). The presence of positive local spatial genetic structure can be measured using Mantel tests (Smouse *et al.* 1986) and spatial autocorrelation analysis (Epperson & Li 1996). The combination of microsatellite markers and multivariate spatial autocorrelation methods such as the multilocus, multiallele method of Smouse & Peakall (1999) are very sensitive in detecting unexpected fine-scale genetic structure. Spatial autocorrelation analysis has also been used to detect sex differences in dispersal in males and females in such diverse species as the chinook salmon (*Oncorhynchus tshawytscha;* Neville *et al.* 2006), the superb fairy-wren (*Malurus cyaneus;* Double *et al.* 2005), the white-breasted thrasher (*Ramphocinclus brachyurus*; Temple *et al.* 2006), Cunningham's

skink (*Egernia cunninghami*; Stow *et al.* 2001), and the slatey-grey snake (*Stegonotus cucullatus*; Dubey *et al.* 2008). In contrast, a lack of sex differences in dispersal has been shown in white-winged choughs (*Corcorax melanorhamphos*; Beck *et al.* 2008) and the tuatara (*Sphenodon punctatus*; Moore *et al.* 2008).

We used a multivariate and multilocus form of spatial autocorrelation analysis (Smouse & Peakall 1999) to investigate sex-biased dispersal and natal philopatry in the diamondback terrapin (*Malaclemys terrapin*). We compared results from spatial autocorrelation analyses with assignment methods and Wright's F_{ST} estimators computed both for males and females. Additionally we compared results of indirect measurements of dispersal with the direct method of measuring movements through capture-mark-recapture.

The diamondback terrapin is a sexually dimorphic turtle species that inhabits the coastal brackish estuaries and marshes along the Atlantic and Gulf coasts of the United States, from Corpus Christi, Texas to Wellfleet, Massachusetts (Iverson 1992). Nesting ecology and population studies indicate that terrapins exhibit nest site fidelity (Auger 1989; Mitro 2003; Roosenburg 1996) and high fidelity to specific creeks (Gibbons *et al.* 2001) or sections of a river (Roosenburg *et al.* 1999). Capture-mark-recapture studies show male and female terrapins occupying areas within a few meters of their original capture location for up to 16 years (Gibbons *et al.* 2001; Lovich & Gibbons 1990), which is presumed to represent the total lifetime adult movement in terrapins (>80 mm plastron length, >3 years old). The generation time is unknown for this species but related species have a generation time of 10 years

(*Chrysemys picta*; (Wilbur 1975) and 37 years (*Emydoidea blandingii*; (Congdon *et al.* 1993).

Average yearly home ranges vary from 0.54 km^2 to 3.05 km^2 (Butler unpublished; Spivey 1998) and average movement is 750.6 m over a period of six days (range 440-1160 m; Harden *et al.* 2007). Nesting ecology studies indicate that nesting female terrapins return to the same dune area to nest (Burger 1977) and nest within 203 m of their original nest site over two nesting seasons (Szerlag & McRobert 2007). There are a few occurrences of longer distance movements in which nesting female terrapins moved roundtrip from marsh areas to nesting areas (roundtrip 4-10 km; Butler unpublished; Gibbons *et al.* 2001). No studies have documented females nesting on their natal beaches. Although strong data for site fidelity exist in demography studies, low to no genetic differentiation within eastern North Carolina (Hart 2005), southwestern Florida (Hart 2005), South Carolina (Hauswaldt & Glenn 2005), and central New Jersey (Chapter 2) suggest significant gene flow. The objective of this study was to (1) determine if sex-biased dispersal occurs in the diamondback terrapin, (2) determine if natal philopatry occurs in the diamondback terrapin, and (3) compare indirect methods of measuring dispersal with direct methods of measuring movements within home ranges and dispersal between study sites within a four year period.

3.3 Methods

Study site and field sampling methods

The study site, the Barnegat Bay Estuary, is a 70 km long estuary located along the central coast of New Jersey. Barnegat Bay is ecologically threatened by changes in water quality and quantity, habitat loss and alteration, and fisheries decline from anthropogenic activities (BBNEP 2002). We trapped terrapins between June-September in 2006-2009 at several locations (Figure 3-1). At Island Beach State Park (IBSP), we trapped individuals of both sexes in a tidal creek and nesting females on Sedge Island (SI) during the nesting season. At the Lighthouse Center (LHC), we trapped individuals of both sexes in man-made tidal canals and nesting females along a dirt path adjacent to the canals. At North Forsythe (NF), we trapped individuals of both sexes in approximately nine tidal creeks and nesting females on Conklin Island (CI) during the nesting season. In South Forsythe (SF), we trapped individuals of both sexes in several tidal creeks. At West Creek (WC), we captured nesting females along the dirt embankment alongside a road running adjacent to the tidal creek. Finally, at Great Bay (GB) we trapped individuals of both sexes in the tidal creeks adjacent to a road spanning through the marsh complex and nesting females along the dirt embankment alongside the road during the nesting season. We trapped terrapins using hoop nets, fyke nets, dip nets, and hand capture. We individually marked turtles by notching the shell (Cagle 1939) and sexed them (Tucker *et al.* 2001). We took blood samples via the subcarapacial sinus (Hernandez-Divers *et al.* 2002) or the brachial artery (Avery & Vitt 1984). We collected some samples from dead animals from tail

tissue or scutes. These samples were from females killed on the road during the nesting season at GB and were collected within 24 hours and several meters vehicle encounter. We recorded additional information such as GPS location, mass, straight carapace length, width, height, and plastron length.

Direct analyses of movement and dispersal

We used capture data from the North Forsythe (NF) study area exclusively for the analysis of movement because it was the only site that was trapped consecutively for four years (2006-2009), contained a large area (~9 km^2), and was trapped consistently each year from June-September. We briefly compared NF movements to those reported in the longest reported mark-recapture analysis for the terrapin (Gibbons *et al.* 2001) and to a smaller dataset of individuals recaptured in the SF study area.

To evaluate differences in movements between male and female terrapins, we calculated each measure of movement for males and females separately. Because individuals often disperse at the onset of sexual maturity (Handley & Perrin 2007), we classified individuals into 4 maturity/sex classes: sexually mature males (MM), sexually mature females (MF), juvenile males (JM), and juvenile females (JF). We determined sex on the basis of carapace length, tail thickness, and cloacal positioning (Tucker *et al.* 2001). We considered sexually mature females to be females with a straight carapace length of 140 mm or more, based on minimum sizes of gravid females at our study site. We considered sexually mature males to be those greater

than 300 g (Roosenburg 1990). All other individuals that were below these minimum values were considered as juveniles.

Before applying any analyses to the mark-recapture data set in NF, we assessed how well the capture histories met the following necessary assumptions of the classic Cormack-Jolly-Seber (CJS) open population model. (1) Every animal has the same probability of recapture. (2) Every marked animal has an equal probability of survival. (3) No marks are lost or overlooked. (4) Sampling is instantaneous and all individuals are released immediately after sampling. Assumption 3 was met because our method of marking turtles with shell notches provides a permanent and unambiguous mark (Cagle 1939). In the four years of study we did not observe any wear around the notches that would cause them to be misread. Marks were further verified because mature females were notched and also injected with a passively induced transponder (PIT) tag (Biomark). We did not record any instance where a recaptured female's PIT tag did not match her assigned notch code. Assumption 4 was met because we released all individuals at the location of capture within 24 hours.

We used goodness of fit testing to determine whether assumptions 3 and 4 were met. We used the program RELEASE (Burnham *et al.* 1987) within the program MARK (White & Burnham 1999) for the NF study area. RELEASE implements goodness of fit testing (Burnham *et al.* 1987) to test for homogeneity in survival and recapture rates (TEST3) and independence of captures (TEST2). Generally TEST2 is indicative of a "trap effect" or other natural phenomena that mimic genuine trap

dependence (Pradel 1993). By testing for different probabilities of subsequent recapture between new individuals and previously identified individuals captured in the same sampling period, TEST3 is indicative of the effects of transients (Burnham *et al.* 1987; Pradel *et al.* 1997). First, we analyzed the capture data from NF (2006-2009) without separating the data into maturity/sex classes. If the data failed the overall goodness of fit tests, we proceeded to separate the data into groups on the basis of sex and subsequently into groups based on maturity. We paid particular attention to TEST3.SR because it compares for each occasion in succession the fates (seen gain or not seen again) of animals entering the experiment (on a given occasion) with those of the animals previously captured. This test is the most applicable test in RELEASE for the detection of transients or individuals that dispersed from the study site (Pradel *et al.* 1997).

We calculated the average and maximum distances moved by each individual between 2006-2009. We used an ANOVA to determine if the average distance between recaptures was significantly greater for one maturity/sex class. Any difference in the average distance from the original capture site and recapture sites might be due to a tendency for one sex to have larger home ranges, rather than genuine dispersal. To assess whether individuals were dispersing further away from their original capture site over time, we tested if the distance between an individual's original capture and its recapture site(s) significantly increased with the number of days between capture events (*sensu* Dubey *et al.* 2008).

In the NF study area we also compared the sex ratio of unmarked individuals captured in 2009 with the sex ratio of individuals captured from 2006-2008 to determine whether one maturity/sex class over-represented the new captures in 2009. We assume that unmarked individuals are of potentially mixed origin (i.e. they may reside in the NF study area but were not captured or they could be immigrants, either permanent or transient, from another area outside of the NF). Any sex bias in dispersal might be reflected in the composition of the sample from 2009. For example, if dispersal is primarily by males, then we should find relatively larger numbers of unmarked males in the study area (Dubey *et al.* 2008) in 2009 because dispersing males from outside the study area would be more likely to be found inside the study area than non-dispersing females.

DNA laboratory procedures

We preserved blood samples on FTA® cards (Whatman, Part of GE Healthcare) at room temperature away from direct heat, moisture, and UV exposure. We followed standard manufacturer's instructions for disc removal and modified procedures for FTA purification (Whatman). We punched a 1.2 mm disc (Harris Micro-Punch) from each card using a cutting mat. To prevent cross contamination between punches, we rinsed the cutting mat with ethanol between each sample. We also punched a disc from an unused FTA® card between each sample to prevent cross contamination on the micro punch. We placed the sample disc in a PCR amplification tube. We rinsed samples once with 50 μl of 70% ethyl alcohol for 5 minutes, twice

with 50 μl of FTA® purification reagent for 5 minutes, and twice with 50 μl of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) for 5 minutes. We dried sample discs for approximately 10-15 minutes on a heating block at 50^{o}C. We conducted PCR analysis within 3 hours of disc washing.

We froze tissue or scute samples at -20^{o}C. We rinsed tissue samples twice with 200 μl of 1x Phosphate Buffer Saline Solution (1x, pH 7.4, 11.9mM Phosphates, 137mM Sodium Chloride, and 2.7 mM Potassium Chloride) prior to extraction. We extracted genomic DNA with the DNeasy Tissue Kit, following the manufacturer's instructions (QIAGEN).

We designed a 6-microsatellite loci protocol for polymerase chain reaction (PCR) and sequencer load multiplexing. We selected loci from the 27 microsatellite loci that were originally developed for the bog turtle (*Glyptemys muhlenbergii*; King & Julian 2004). King and Julian (2004) screened the 27 loci in *Malaclemys terrapin* and found that 23 successfully amplify and 16 exhibit moderate to high polymorphism. Hart (2005) further screened the 16 loci and chose 12 based on levels of polymorphism and ease of use. Using allele frequencies at each of the 12 loci sampled in Cape May, NJ and Sandy Hook, NJ (Hart 2005), we selected 6 microsatellite loci for this study based on levels of polymorphism, PI, and PI_{sib} (Waits *et al.* 2001). Selected primers and details of the multiplex PCR reaction are shown in Table 3-1**.** We ran multiplex PCR products on an ABI 3100 capillary sequencer (Applied Biosystems) together with the internal size standard GENESCAN 500 ROX

(Applied Biosystems). We conducted fragment analysis using the software Peak Scanner version 1.0 (Applied Biosystems).

Genetic diversity analyses were conducted using all DNA samples collected from all six study locations (Figure 3-1). We used MICROCHECKER 2.2.3 (Oosterhout *et al.* 2004) to detect any genotyping errors, extreme stuttering, and null alleles. We calculated null alleles using a Monte Carlo simulation with bootstrap method to generate expected homozygote and heterozygote allele size difference frequencies which were then compared to the expected Hardy-Weinberg frequencies. We calculated genetic diversity measurements such as mean number of alleles per locus, allele frequencies, and gene diversity (Nei 1987) using FSTAT 2.9.3 (Goudet 2001). We used GenALex (Peakall & Smouse 2006) to calculated observed, H_O, and expected, H_E, heterozygosities. We tested for significant deviations from Hardy-Weinberg equilibrium and the presence of linkage disequilibrium using FSTAT 2.9.3 (Goudet 2001), with strict Bonferroni correction applied for multiple comparisons (Rice 1989). We used GenALex (Peakall & Smouse 2006) to calculate probability of identity, PI, an estimate of the average probability that two unrelated individuals will by chance have the same multilocus genotype and PI_{sibs}, a probability of identity that takes into account the genetic similarity among siblings (Waits *et al.* 2001).

Genetic analyses

Because direct measurements of movement and dispersal do not necessarily indicate gene flow, we conducted seven indirect (i.e. genetic) tests for biased

dispersal using subpopulations groupings IBSP, NF, and SF. These three locations included adequate DNA sampling of each maturity/sex class (Table 3-2). The distance between these sites is 9.6 km (IBSP-NF), 13.6 km (IBSP-SF), and 4.1 km (NF-SF). We choose these three populations because we genotyped sufficient numbers of individuals of each maturity/sex class for comparison. We did not utilize LHC, WC, and GB for this analysis since sampling at these locations was biased towards nesting females and thus resulted in low sample sizes for the remaining three maturity/sex classes. The seven indirect tests for biased dispersal included: (i) mean corrected assignment index (*m*AIc), (ii) variance of assignment index (*v*AIc), (iii) F_{IS}, a measure of inbreeding within subpopulations relative to the total, (Weir & Cockerham 1984), (iv) F_{ST}, genetic differentiation among subpopulations (Weir & Cockerham 1984), (v) average pairwise relatedness (r), (vi) first generation migrant tests, and (vii) spatial autocorrelation.

We calculated *m*AIc, *v*AIc, F_{IS}, $F_{ST,}$ and average relatedness in FSTAT 2.9.3 (Goudet 1995). We determined statistical significance by comparing actual values to randomized values for 10,000 permutations. Assignment indices followed methods of Paetkau *et al.* (1995) which were later modified by Favre *et al*. (1997) to calculate corrected assignment values (AIc). We expected individuals of the dispersing sex to have a lower *m*AIc, because immigrants have lower AIc values than residents. We expected individuals of the dispersing sex to have a higher *v*AIC because members of the dispersing sex have both immigrants and residents (Goudet *et al.* 2002). The F_{IS} statistic measures how well the genotype frequencies fit Hardy-Weinberg

expectations (Hartl & Clark 1997). We expected individuals of the dispersing sex to have a higher F_{IS} (heterozygote deficit) than the philopatric sex because of the Wahlund effect (Goudet *et al.* 2002). The F_{ST} statistic describes the proportion of the total genetic variance found among populations (Hartl & Clark 1997). We expected individuals of the philopatric sex to have a higher F_{ST} than the dispersing sex because allele frequencies between subpopulations for individuals of the dispersing sex should be more similar due to effective dispersal (Goudet *et al.* 2002). Average relatedness can be calculated from F_{ST} and F_{IT} through a simple equation [$r = 2 F_{ST} /(1+ F_{IT})$; Queller & Goodnight 1989 estimator implemented in FSTAT 2.9.3]. We expected individuals of the dispersing sex to have a lower average relatedness within locations when compared to the philopatric sex (Prugnolle & de Meeus 2002). We ran analyses with males and females regardless of maturity, mature males and females, and juvenile males and females with three population groupings (IBSP, NF and SF).

First generation (F_0) migrant tests (GENECLASS 2.0; Piry *et al.* 2004) were used to determine F_0 migrants among mature individuals sampled in IBSP, NF, and SF. For migrant detection, we used a likelihood computation with a default frequency for missing alleles of 0.01 and L = L home, the likelihood of the individual genotype within the population where the individual has been sampled (Paetkau *et al.* 1995). This method performs better than others if some potential source populations were not sampled (Piry *et al.* 2004). The likelihood computation mirrored the calculations used in our assignment tests in FSTAT 2.9.3, with the exception that rather than only reporting the mean (*m*AIc) or variance (*v*AIc) of likelihood values for each sex,

GENECLASS 2.0 reports individual likelihood values. We computed the probability that an individual is a resident by using a Monte Carlo resampling algorithm (Paetkau *et al.* 2004) with 10,000 simulated individuals and Type I error rate of 0.05. We tested for differences in the total number and the proportion of male and female F_0 migrants.

We used the program GENALEX version 6.2 (Peakall & Smouse 2006) to estimate the spatial extent and magnitude of positive correlation among individual multilocus genotypes across the landscape. GENALEX calculates the multilocus autocorrelation coefficient *r* among individual genotypes falling within various distance classes. We calculated the *r* correlation coefficient using two pairwise matrices, one using geographic distances and the other using squared genetic distances (Smouse & Peakall 1999). The *r* correlation coefficient is similar to Moran's I coefficient, ranges from -1 to +1, and provides a measure of genetic similarity between pairs of individuals falling within each distance class (Peakall *et al.* 2003). To test whether the r correlation coefficient was significantly different from the null hypothesis of no spatial genetic structure, we performed 1,000 random permutations to determine upper and lower confidence intervals for the null hypothesis (Peakall *et al.* 2003)**.**

We presented the correlation coefficient *r* and associated confidence interval about r = 0 as correlograms, which displayed *r* in relation to distance. We divided distance on the correlogram into user specified bins. We used bin sizes which represented both the spatial resolution within sampling locations (500, 1000, and

2000 m) and the spatial resolution between sampling locations (4000, 8000, 14000 m) for the dataset comprised of IBSP, NF, and SF individuals. Within a given correlogram, we also present the number of pairwise comparisons used at each distance class and the p-values for the one-tailed probabilities of positive and negative spatial genetic structure. We determined distances where the one-tailed probabilities of no spatial genetic structure indicated a significance of $P < 0.05$.

To evaluate whether natal philopatry of nesting females occurred, we estimated *r* (as a function of distance) for nesting females (N=132) from 5 nesting areas (GB, n=84; WC, n=9; SI, n=33; CI, n=12; LHC, n=5). For some nesting females we had exact GPS coordinates of the location where the nest was deposited. For females captured on a nesting beach prior to nesting, we assumed that the female would nest at the capture location. We used distance classes which represented both the spatial resolution within sampling locations (5, 500, 1000, and 5000 m) and the spatial resolution between sampling locations (10,000, 20,000, 36,000 m) for the dataset comprised of GB, WC, SI, CI, and LHC nesting females.

The spatial extent of significant autocorrelation may change when evaluating different distance classes, because the observed patterns are the composite of the true spatial extent and the number of individuals that are evaluated at each distance class (Peakall *et al.* 2003). Therefore, we also evaluated composite graphs containing the results from the first distance bin by using different bin sizes. This method evaluated how data pooling affected autocorrelation and allowed us to assess the true extent of detectable positive autocorrelation (Double *et al.* 2005).

3.4 Results

Direct analyses of movement and dispersal

We marked and released a total of 1277 diamondback terrapins over the four year study period (2006-2009) in North Forsythe (JF = 231; MF = 579; JM = 313; MM = 136; and juvenile/no sex, N = 18). Recapture rates were relatively low between years, with 147 individuals recaptured at least once, 18 individuals recaptured at least twice, and 1 individual recaptured three times. The percent of individuals recaptured at least once in a later year was 11.5%. There were no terrapins initially marked within NF captured at other study locations, however there were two mature females marked several years prior at the SI nesting location that were recaptured in NF in 2009. The first female was marked and captured nesting three consecutive years on SI from early June-mid July as part of another study in 2002, 2003, and 2004 (Wnek, personal communication) and was then recaptured non-gravid in a fyke net 8.5 km away in NF in early July of 2009. The second female was captured nesting on SI in early June of 2002, 2005, and 2007 and then captured gravid in a hoop trap 8.1 km away in NF in early July of 2009.

Output from program RELEASE indicated the goodness of fit tests (TEST2 + TEST3, combined) were significant when the data was pooled for all maturity/sex classes ($P = 0.0013$), indicating heterogeneity in survival and capture probabilities (Burnham et al. 1987). Further examination of the results indicated that TEST2 ($P = 0.4291$) and TEST3.SM ($P = 1.0$) were not significant, but TEST3.SR failed the goodness of fit test ($P < 0.001$). When the data were grouped according to sexes, the

overall goodness of fit test (TEST2 + TEST3, combined) was not significant for females ($P = 0.21$), but males failed the overall goodness of fit test ($P = 0.012$). Further examination of the results for males indicated that TEST2 ($P = 0.68$) and TEST3.SM ($P = 0.79$) were not significant, but males significantly failed TEST3.SR ($P < 0.01$). Because the data set included four trapping occasions (2006-2009), two occasions could be analyzed for TEST3.SR. On occasion 2 (i.e. 2007), males did not fail TEST3.SR ($P = 0.33$). On occasion 3 (i.e. 2008), males significantly failed TEST3.SR ($P < 0.001$). The contingency table for this test indicated fewer individuals that were caught, newly marked, and released on this occasion were seen again (4.8%) when compared to individuals that were caught on this occasion, marked before, released, and seen again in a subsequent occasion (30%). This implicates the possibility that some males marked on this occasion were transient individuals. When the male dataset was grouped according to maturity, the overall goodness of fit test for mature males was not significant ($P = 0.334$) nor were any of the subtests significant ($P > 0.12$). The overall goodness of fit test for juvenile males was marginally non-significant ($P = 0.08$). TEST2, TEST3.SM2 and TEST3.SR occasion 2 were not significant ($P > 0.73$). However, the contingency table for TEST 3.SR occasion 3 ($P = 0.004$) significantly indicated that that fewer individuals that were caught, newly marked, and released on this occasion were seen again (4.5%) when compared to individuals that were caught, marked before, and released on this occasion (33%). Thus, implicating that male transients were likely juvenile individuals.

The maximum distance a terrapin moved was 1984 m by a mature female (Table 3-3); excluding the data from the two mature females marked on SI in 2002 and recaptured in NF in 2009 because they appeared to be rare dispersal events. We found significant differences in average movements between immature and mature males and females (ANOVA, $F_{3,143} = 8.67$, $P < 0.001$). Post-hoc Tukey-Kramer tests detected that mature females had moved greater distances than juvenile males and mature males and that juvenile females moved greater distances than mature males (at the 0.05 level of significance). The distance between the original capture site and all subsequent capture sites did not increase with time (days) for any maturity/sex class (MF: $F_{1,63} = 2.93$, $P = 0.09$; MM: $F_{1,47} = 0.001$, $P = 0.98$, JF: $F_{1,60} = 0.38$, $P = 0.54$, JM: $F_{1,81} = 1.29$, $P = 0.26$).

In the SF sampling location, we captured 169 individuals over the three year trapping period (2007-2009). Twenty individuals were recaptured once and five individuals were recaptured twice. Trapping efforts in SF typically occurred from the last week in July to the first week in September, and therefore missed any movements associated with activities occurring in June and early July, such as nesting. Sample sizes of some maturity/sex classes were low (JF = 8, JM = 1, MF = 14, MM = 2), therefore we pooled the data to determine average movement (21 m) and maximum movement (227 m). Distance between sampling locations did not increase as a function of time ($F_{1,33} = 0.25$, $P = 0.62$).

Females comprised most of the 247 new captures (53%) in 2009. Juvenile females, juvenile males, and mature males comprised 17%, 20%, and 10%

(respectively) of the new captures in 2009. New captures in 2009 made up 18% of total captures of adult males, 22% of total adult females, 16% of total juvenile males, and 18% of total juvenile females. New captures in 2009 did not account for a larger proportion of any one maturity/age class ($\chi^2 = 6.02$, d.f. = 3, P = 0.11). New captures can consist of both unmarked residents and immigrants and it is likely that our resident population in NF is quite large (>4,000 Sheridan and Avery, unpublished data). Even when a large majority of the resident population has been captured, it might be difficult to distinguish whether differences in sex ratios of new captures are due to environmental sex determination (ESD; Jeyasuria *et al.* 1994), increased mortality, or sex-biased dispersal unless hatchling sex ratio and mortality rates are also estimated.

Genetic analysis

We found 74 different alleles in the 1558 individual males, females and juveniles that were genotyped (DNA samples collected 2006-2008 from all trapping locations). Across the six targeted loci, the number of alleles per locus ranged from 10 (*Gmu*D114 and *Gmu*D90) to 17 (*Gmu*D121), with a mean of 12.33 alleles per locus. Mean expected heterozygosity (H_E) and observed heterozygosity (H_O) across the study area were 0.808 and 0.825, respectively. There was a low probability of individuals sharing an identical genotype ($PI = 1.4 \times 10^{-08}$ and $PI_{sibs} = 1.9 \times 10^{-03}$). After strict Bonferroni correction (Rice 1989), no departures from Hardy-Weinberg equilibrium were detected at any loci (within study sites and over all study sites).

Possible null alleles were detected for locus *Gmu*D121, however, this only held true for the site GB and not for other sampling sites, or across all sites. The null allele frequency at the *Gmu*D121 locus in GB samples was 0.041 (Chakraborty *et al.* 1992) and 0.037 (Brookfield 1996). The lack of departures from Hardy-Weinberg equilibrium indicated the effect of possible null alleles in *Gmu*D121 was limited. Therefore, we included all loci in our data set. No linkage disequilibrium was detected for any pair of loci at any sampling site after strict Bonferroni correction [a = 0.05, k = 90, $p < 0.001$; (Rice 1989)].

When testing for sex-biased dispersal among all males and females genotyped in NF, SF, and IBSP, we found no significant sex-biased dispersal (Table 3-4a). Mean assignment index, variance of assignment index, F_{ST}, F_{IS} and average pairwise relatedness were similar in males and females (Table 3-4a). However, we did see evidence for some male-biased dispersal when we limited the analysis to mature males and mature females (Table 3-4b), as the mean assignment index was lower for males than females (Table 3-4b). The variance of assignment index, F_{ST}, F_{IS} and average pairwise relatedness, however, did not differ in mature males and females (Table 3-4b). Finally, there was no sex-biased dispersal among juvenile males and juvenile females (Table 3-4c).

First generation assignment tests ($\alpha = 0.05$; GENECLASS 2.0) of mature individuals identified a total of 32 individuals as F_0 migrants (10 MM and 22 MF). Ten F_0 migrants were captured in IBSP (6.7% of 150 captures), six of which were assigned to NF, two to SF, and two were home-assigned. A home-assigned migrant

indicates that this individual was genetically closer to its population of capture than to other sampled populations and that it may have been assigned to another population if we had collected data from additional populations. Individuals can be home-assigned Eighteen F_0 migrants were captured in NF (3.5% of 512 captures), eight of which were assigned to SF, three to IBSP, and seven were home-assigned. Four F_0 migrants were captured in SF (6.5% of 62 captures), three of which were assigned to NF and one was home-assigned. Overall, 4.4% (32 of 724 captures) were identified as F_0 migrants. The proportion of individuals assigned as F_0 migrants did not differ between the three trapping locations (χ^2=3.39, df=2, P=0.18). Overall, a larger number of MF were identified as F_0 migrants when compared to MM (χ^2=4.5, df=1, P=0.034). Although our ability to determine the overall tendency for one sex to disperse more than another could be affected by the larger number of MF (604 vs. 119 MM, P< 0.001). When the larger proportion of MF compared to MM was taken into account, the proportion of immigrants vs. residents was significantly greater for MM (8.4%) than MF (3.6%; χ^2=5.33, df=1, P=0.021).

Spatial autocorrelation analysis of all individuals genotyped from IBSP, NFOR, SFOR (N = 1300) showed significantly positive *r* values within the 1,000-2,000 m distance class and significantly negative r values within the 8,000-14,000 m distance class (Figure 3-2a). Near-identical results were found when the analyses were restricted to mature females (Figure 3-2b). The differences were that, for mature females only, the *r* value for the 1,000-2,000 m distance class was >2x larger (0.0023 vs. 0.0009), the r value for the 8,000-14,000 m distance class was ~ 2x smaller (-

0.0017 vs. -0.0008), and there was a significantly positive r value at the 500-1,000 m distance class. When evaluating the effect of bin size, mature females showed significantly positive *r* values for the 0-2000, 0-5000, and 0-7500 m distance class sizes, but not for the 0-10000 m bin size. For those three bin sizes, the x-intercepts were from 3,415, 7,267, and 10,201 m. In contrast, when analyses were restricted to mature males only (Figure 3-2c), juvenile males only (not shown), and juvenile females only (not shown), the spatial autocorrelation analysis showed no significant positive autocorrelation. For mature males, a significantly negative *r* value was found within the 1,000-2,000 m distance class (Figure 3-2c). For juvenile males, no significant values were found within any of the distance classes (not shown). For juvenile females, a significantly negative *r* value occurred within the 500-1,000 m distance class (not shown).

Spatial autocorrelation analysis of all nesting females (N = 143) showed significantly positive *r* values within the 0-50 m distance class (Figure 3-2d). When evaluating the effect of bin size, nesting females had significantly positive *r* values for the 0-5 (r = 0.013), 0-10 (r = 0.013), 0-50 (r = 0.015), 0-100 (r = 0.012), and 0-150 m (r = 0.014) bin sizes and the x-intercepts were 19, 57, 67, 183, 201 m (respectively).

3.5 Discussion

The contrast in movements and dispersal measured via direct measures and dispersal measured via indirect genetic measures in males in this study highlights the

limitations of detecting long-distance or infrequent dispersal using direct methods (Slatkin 1985). Our direct observational method of measuring movement indicated that all individuals have moved relatively small distances (<2000 m; Table 3-3, with the exception of 2 MF dispersing greater than 8km). The distances are similar to studies that show high fidelity of terrapins to marsh areas (Gibbons *et al.* 2001; Harden *et al.* 2007; Roosenburg *et al.* 1999) and with small home ranges (Butler unpublished; Spivey 1998). Furthermore, the direct methods indicated that within these sampling locations, mature females moved greater distances than males (Table 3-3). Because distance moved was not correlated with time in any of the maturity/sex classes, we conclude that direct measurements of movement indicate simply that females simply have greater home range sizes than males (*sensu* Dubey *et al.* 2008). Finally, our goodness of fit analysis of our mark-recapture study indicated that some juvenile males were likely transient individuals dispersing through the NF location, even though we had no records of these individuals captured in another sampling location. Other the other hand, our mark recapture documented the dispersal of two nesting females from the Sedge Island sampling location to North Forsythe.

There was no significant positive autocorrelation for mature males at any distance class, inconsistent with the maximum movements measured by direct methods (< 800 m; Table 3-3). Genetic autocorrelation suggested the possibility that mature females were randomly dispersing from 0-500 m but then aggregating at distances 500-2,000m. Although significantly different from zero the r values were very low (≤0.0023) and thus may not indicate any biological significance. The

number of pairwise comparisons (n> 20,000) was quite high and increased the possibility that any deviation from r = 0 would be significant. On the other hand, for nesting females we detected significantly positive genetic autocorrelation (r = 0.015) from 0-50 m (maximum spatial extent 201 m) with a smaller number of pairwise comparisons (n = 636). The spatial autocorrelation of nesting females indicates that females are exhibiting natal philopatry to nesting beaches and the data are in agreement with a mark-recapture study that document average distances between a female's nests sites of 203 m (Szerlag & McRobert 2007).

Although, r was more than 6x higher for nesting females (r = 0.01), the correlation coefficient was still quite low considering full-siblings are expected to have a relatedness of 0.50. Several factors could play a role in slowing the accumulation of relatedness between philopatric females on a section of a nesting beach including: breeding group size, mean and variance in the number of successful progeny produced with each mating, mating system, and effective movement of individuals between nesting locations (Scribner & Chesser 2001). Average coancestries (θ; Chesser 1991) of individuals on a nesting area can be affected by the number of nesting females and the number of successful offspring produced by each female. Given the large number of individuals captured (and low recapture rate), our nesting populations could potentially produce a large number of successful clutches each year; thereby slowing the accumulation of coancestry within nesting areas. Multiple paternity within clutches can also reduce the level of relatedness within clutches to that of half-siblings (r = 0.25) compared to clutches with single paternity

(r = 0.50). Multiple paternity is common in *M. terrapin,* with 19.0% to 31.4% of clutches from nesting beaches in Barnegat Bay, NJ exhibiting multiple paternity (Chapter 4). Lastly, if males effectively disperse between nesting populations, if females with home ranges extending into adjacent populations effectively mate with males from adjacent populations, or if some females do not exhibit strict natal site philopatry, then the accumulation of coancestry on natal beaches could be reduced.

Both our mAIc and F_0 migrant tests indicated dispersal is male-biased, but that *per capita* there are more female than male dispersers in these populations with female-biased sex ratios. Potentially some female F_0 migrants may be foraging or mating in the area where they were captured and they may seasonally return to their natal beach to lay eggs (rather than nesting in the location where they were captured). Researchers have documented roundtrip movements of nesting females from marsh areas to nesting areas (roundtrip 4-10 km; Butler unpublished; Gibbons *et al.* 2001) and with documentation of nesting site fidelity (Auger 1989; Burger 1977; Mitro 2003; Roosenburg 1996). Female natal homing was also recently documented in the closely related freshwater turtle species, *Graptemys kohnii* (Freedberg *et al.* 2005). The majority of *G. kohnii* females returned to nesting locations within 160 m of their initial nesting location and females readily returned to their original nesting area even after being transplanted up to 6 km away (Freedberg *et al.* 2005). Despite documentation of nest site fidelity and natal philopatry, we cannot exclude the possibility that some F_0 females are nesting on non-natal beaches. Indeed, three of the 33 SI nesting females included in the GENECLASS migrant tests were considered F_0

migrants. Two of the females appeared to exhibit nesting site fidelity, while the third was only captured one time. The first was documented nesting on SI in four separate years (2002, 2004, 2006, and 2007) and the other was documented nesting two years (2006 and 2007). It is unknown whether these females failed to nest in the years when they were not detected on SI, if they nested elsewhere, or if they nested on SI but were not detected.

Despite the low natal philopatry in a handful of the females sampled, our spatial autocorrelation analysis indicates that many females are philopatric to natal beaches. Female natal philopatry might be favored by selection because it ensures that a female nests at a location that successfully hatched offspring in the previous generation, thereby increasing lifetime fitness (Freedberg & Wade 2001; Reinhold 1998). In addition, natal philopatry in reptiles with environmental sex determination (ESD) may explain why sex ratios are biased towards females (Freedberg & Wade 2001). The Freedberg and Wade's (2001) model of ESD coupled with natal philopatry demonstrates that when a nesting area is inherited maternally, a maternal lineage that produces an excess of daughters will be favored over maternal lineages producing an excess of sons.

In our study, the *m*AIc test detected the presence of sex-biased gene flow in mature males, while F_{ST}, F_{IS}, *v*AIc, and average pairwise relatedness (r) did not detect any sex-biased gene flow. Mathematical simulations show that these tests differ in their sensitivity in relation to various parameters, such as dispersal rate, bias intensity, number of individuals sampled, and number of loci sampled (Goudet *et al.* 2002). The

F_{IS} statistic has very low sensitivity in all cases (<70%; Goudet *et al.* 2002). The F_{ST} statistic (and the associated average pairwise relatedness) performs best at higher dispersal rates (>10%), *v*AIc performs best at low dispersal rates (<10%), and *m*AIc is intermediate between the two tests (Goudet *et al.* 2002). It is important to note that *v*AIc and F_{ST} also performed poorly in detecting sex-biased dispersal in terrapins from Carteret County, North Carolina, but *m*AIc significantly detected male-biased dispersal (Hart 2005). Both mAIc and vAIc detected male biased dispersal in the Florida Everglades where there was a male biased sex ratio was 1.0:1.2 (Hart 2005). Given that the *m*AIc test detected sex-biased gene flow, we suggest that the inability of F_{ST}, F_{IS}, *v*AIc, and average pairwise relatedness (r) to detect sex-biased gene flow may be due to several parameters (e.g. small number of loci sampled and bias intensity) rather than a true lack of sex-biased gene flow.

Our comparisons of tests of sex-biased dispersal between male-female (all size classes combined), mature male-mature female, and juvenile male-juvenile female, highlight the importance of analyzing cohorts (or life stages) separately (Lawson Handley & Perrin 2007). Analyzing all males and females, regardless of sexual maturity, can reduce the ability of the tests to detect sex-biased dispersal (Prugnolle & de Meeus 2002). Some juveniles were likely the offspring of philopatric females and immigrant males or females mating with males when temporally dispersing into neighboring populations prior to return to natal areas to nest. Since our genetic markers were biparental, alleles were independently assorted in the offspring (Handley & Perrin 2007) and the signature of sex-biased dispersal was removed.

Conservation Implications

Male-biased dispersal and female natal philopatry occurred in the diamondback terrapin. These data have important implications for both females and males. First, loss of nesting beaches could have significant negative impacts on females that attempt to return to non-existent or altered nesting beaches. For example, females attempting to nest in an area that has been recently developed may encounter human activities, such as boat traffic, vehicles, and bulkheading. If females are persistent to nest in these areas they could suffer injury by vehicles (Hoden & Able 2003; Szerlag & McRobert 2006; Wood & Herlands 1997) and motorboats (Gibbons *et al.* 2001; Tucker *et al*. 2001; Avery unpublished), or nest failure. Female terrapins continue return to nest in Margate, NJ, a barrier island with most of its bay front lined with bulkheading, and are usually unsuccessful (Scott, personal communication).

Second, if females cannot find alternative nest locations, then dystocia (egg-binding) may occur. The lack of suitable habitat for nesting often causes female turtles to retain eggs in the oviducts until the environment becomes suitable, both in captivity (Cagle & Tihen 1948; Jackson *et al.* 1971; Miller 1932; Risley 1933) and under natural conditions (Buhlmann *et al.* 1995; Galbraith *et al.* 1988). In some cases, egg retention leads to the movement of eggs into the abdominal cavity via holes eroded in the walls of the oviduct (Cagle & Tihen 1948) or reverse peristalsis (Jackson *et al.* 1971; Risley 1933). Eggs in the abdominal cavity often are infected with bacteria, cause inflammatory reactions (Jackson *et al.* 1971) and could lead to death.

Third, females remaining philopatric to degraded nesting beaches could lay nests with reduced hatching success. Overall, the alteration or loss of nesting beaches could lead to a reduction in the reproductive success, changes in population sex-ratios, and long-term viability of terrapin populations.

Fourth, since males are more prone to disperse than females it is important to reduce threats to males particularly when individuals disperse. Threats to males include boat mortality (Cecala *et al.* 2008; Tucker *et al.* 2001), road mortality (Hoden & Able 2003), crab pot mortality (Bishop 1983; Dorcas *et al.* 2007; Roosenburg *et al.* 1997), pollution (Burger 2002; Basile 2010), and predation (Cecala *et al.* 2008). Maintaining gene flow via males will be particularly important for the conservation of this species, especially in populations with male-biased sex ratios (e.g. Kiawah Island, South Carolina, 1:1.78 male biased; Lovich & Gibbons 1990). Furthermore, it is important to note in areas where extirpation has occurred, males alone cannot reestablish a population (Tucker *et al.* 2001). Thus, although females are less prone to disperse between populations, protection of females during movements to nesting beaches should also be included in conservation plans.

Table 3-1 Characteristics of the 6-microsatellite multiplex kit and measures of gene diversity over all diamondback terrapin samples in Barnegat Bay, NJ (N = 1558).

Loading and PCR Plex	Locus	GenBank Accession #	Primer Concentration (mM)	Size Range (bp)	Label	# of Alleles	H_o	H_e	PI_{sibs}
A	*Gmu* B08	AF517229	0.2	211-241	6-FAM	11	0.829	0.804	0.358
A	*Gmu* D121	AF517252	0.2	124-188	NED	17	0.867	0.876	0.31
A	*Gmu* D62	AF517241	0.25	127-175	HEX	12	0.87	0.794	0.363
B	*Gmu* D87	AF517244	0.2	224-276	6-FAM	14	0.875	0.874	0.32
B	*Gmu* D114	AF517251	0.2	88-124	NED	10	0.696	0.677	0.434
B	*Gmu* D90	AF517247	0.25	111-147	HEX	10	0.81	0.822	0.345

PCR chemistry: 20 µl PCR reactions using 5-15 ng of DNA or 1.2 mm Whatman blood punch, 0.3175 mM dNTPs, 1x GoTaq Flexi Buffer (Promega), 3.75 mM $MgCl_2$, 0.2-0.25 mM primer, 0.5 units of GoTaq polymerase (Promega).

PCR thermocycling: 94°C for 2 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 56°C for 45 s, extension at 72°C for 2 min. Final extension at 72°C for 10 min.

Table 3-2 Samples sizes for mature females, mature males, juvenile females, and juvenile males for which DNA was analyzed from the following sampling locations in Barnegat Bay, NJ: Island Beach State Park (IBSP), North Forsythe (NF), and South Forsythe (SF).

	Mature Females	Mature Males	Juvenile Females	Juvenile Males	Total
IBSP	142	8	35	59	244
NF	408	104	173	251	936
SF	55	7	27	31	120
Total	605	119	235	341	1300

Table 3-3 Mean and maximum distances between captures of diamondback terrapins in Barnegat Bay, NJ that were recaptured up to four times, as measured by direct methods. Data are categorized by sex and maturity.

	N	Mean Distance (m)	SE ± 1	Maximum Distance (m)
Juvenile Females	29	301	46	1379
Mature Females	43	369	59	1984
Juvenile Males	50	148	22	1039
Mature Males	25	104	18	780

Table 3-4a-c Mean assignment (mAIC), variance assignment (vAIC), F-statistics and relatedness (r) for each sex of diamondback terrapins in Barnegat Bay, NJ (4a, all individuals; 4b, mature individuals; 4c, juvenile individuals). Significance (P) was assessed using the randomization method of Goudet et al. (2002). Values in bold indicate P<0.05.

		m AIC	*v* AIC	F_{ST}	F_{IS}	r
a	Males	0.042	5.924	0.002	-0.015	0.004
	Females	-0.024	5.756	0.0001	-0.029	0.0001
	Overall	--	--	0.001	-0.024	0.001
	P	0.683	0.408	0.927	0.061	0.925
b	Males ≥ 30	-0.365	5.723	0.004	-0.020	0.008
	Females ≥	0.072	5.517	0.002	-0.028	0.005
	Overall	--	--	0.002	-0.026	0.004
	P	**0.026**	0.261	0.638	0.301	0.636
c	Males < 30	0.145	6.130	0.004	-0.019	0.007
	Females <	-0.210	6.465	0.002	-0.036	0.004
	Overall	--	--	0.003	-0.026	0.005
	P	0.071	0.336	0.240	0.881	0.245

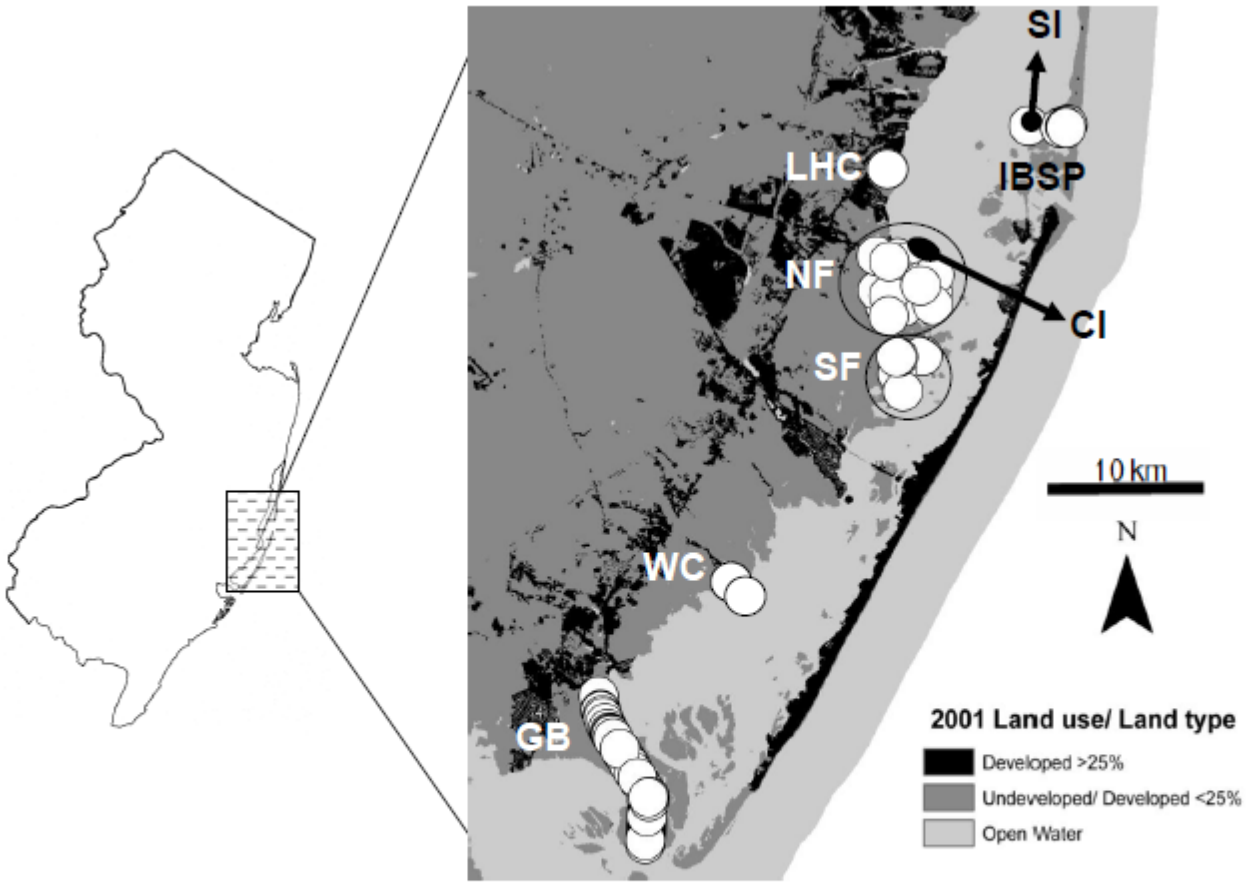
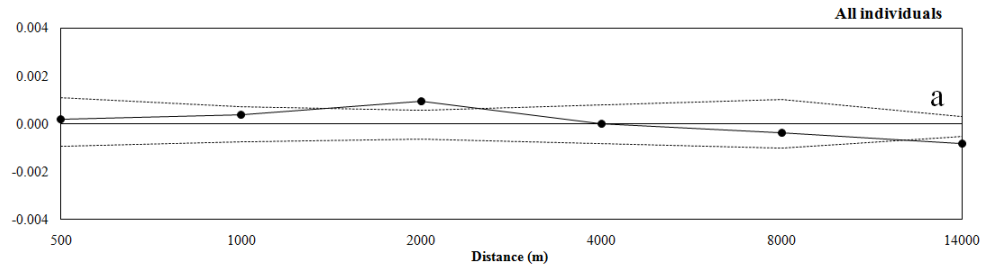
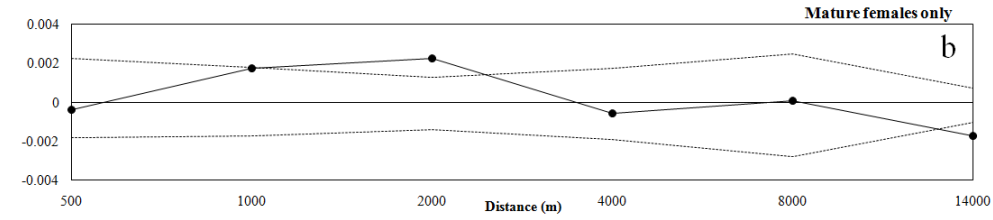


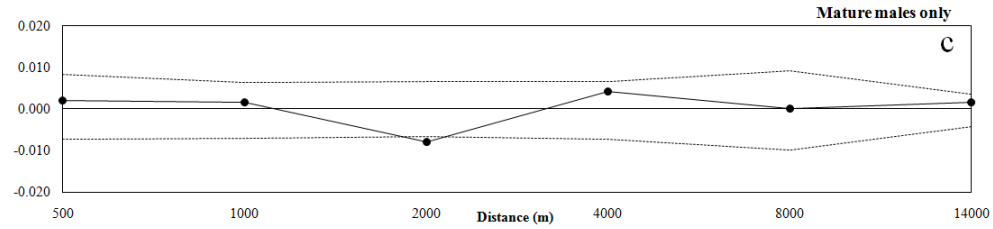
Fig. 3-1 Map of sampling sites on a 2001 land use map. White circles indicate sampling areas at each location, while the two black circles discriminate between North and South Forsythe. Inset indicates general location of study site on a map of New Jersey. Sampling area abbreviations are as follows: Island Beach State Park (IBSP), Lighthouse Center (LHC), North Forsythe (NF), South Forsythe (SF), West Creek (WC), and Great Bay (GB). Locations of Sedge Island (SI) and Conklin Island (CI) are indicated by black arrows.



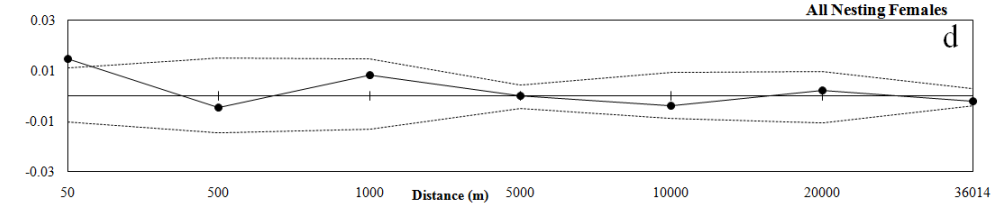
n	107206	128561	184405	113649	58496	247529
P1	0.345	0.164	**0.002**	0.510	0.793	1.000
P2	0.656	0.837	0.999	0.491	0.208	**0.010**



n	22510	24489	38675	22030	10809	62550
P1	0.612	**0.028**	**0.001**	0.755	0.466	0.997
P2	0.389	0.973	1.000	0.246	0.535	**0.004**



n	1308	1456	1483	1399	390	976
P1	0.292	0.333	0.986	0.114	0.544	0.246
P2	0.709	0.668	**0.015**	0.887	0.457	0.755



n	636	435	517	2428	1020	603	4514
P1	**0.008**	0.711	0.134	0.477	0.816	0.354	0.860
P2	0.993	0.290	0.867	0.524	0.185	0.647	0.141

Figure 3-2 Correlogram plots of the genetic correlation coefficient (r) as a function of distance for individuals genotyped and captured in NF, SF, and IBSP (a-c) and SI, LHC, CI, WC, and GB (d) . The permuted 95% confidence interval about the null hypothesis ($r = 0$) is shown. Number of pairwise comparisions (n) for each distance class are shown. P1 is the probability that r-random >= r-data and P2 is the probability that r-random <= r-data, with significant values highlighted in bold.

CHAPTER 4: Inter-population variation of multiple paternity in the diamondback terrapin, *Malaclemys terrapin*

4.1 Abstract

Molecular studies have shown that multiple paternity is common among turtles and that the frequency of multiple paternity varies between species and between populations of the same species. We used nuclear microsatellite markers to compare frequencies of multiple paternity within five nesting locations of the diamondback terrapin (*Malaclemys terrapin*). Using 6 highly polymorphic microsatellite markers, we tested 23 to 46 clutches from five nesting locations and found that the frequency of multiple paternity differed significantly among locations, ranging from 12.5 to 45.7%. Clutches with multiple paternity did not differ from clutches with single paternity with respect to female size, clutch size, egg size, hatchling size, or hatching success, suggesting that multiple paternity may not provide immediate benefits to offspring survivorship. Male and females mated within their home ranges and thus mating events are not responsible for the high levels of gene flow documented in this species. This study also confirmed sperm storage both within and between seasons. The use of stored sperm may play a role in the frequency of multiple paternity. The incidence of multiple paternity exhibited a significant non-linear correlation with population sex ratio, suggesting that it is also likely related to frequency of mating encounters and mate competition, in addition to sperm storage.

4.2 Introduction

Mating systems can affect the level of inbreeding, effective population sizes, and genetic diversity within and among populations. Sperm storage and multiple paternity can increase effective population sizes and genetic variability (Pearse & Anderson 2009; Sugg & Chesser 1994). Advances in molecular techniques, particularly in highly variable microsatellite loci, have made it possible to address sperm storage and mating system strategies in turtles (Alacs *et al.* 2007; FitzSimmons & Hart 2007; Pearse & Avise 2001) whose mating behaviors are extremely difficult to observe in nature. Molecular techniques can identify maternal and paternal components of offspring genotypes and, thus, identify mating behaviors and determine effective population sizes that are critical for conservation and management (Alacs *et al.* 2007), especially in turtle species that are in decline as a result of habitat alteration and fragmentation among other human impacts (Gibbons *et al.* 2000).

Female turtles can store sperm in storage tubules embedded in the oviduct (Gist & Congdon 1998; Gist & Jones 1989) and sperm can be utilized for up to 4 years (fertility declining rapidly after 2 years; Hildebrand 1928). Sperm storage is an important reproductive mechanism that allows females to fertilize consecutive clutches with a single mating event (Fitzsimmons 1998; Kichler *et al.* 1999; Roques *et al.* 2004). Other factors that may select for sperm storage include: asynchronous reproductive cycles of males and females (Birkhead & Moller 1993), an additional

opportunity for mate choice (Eberhard 1998), and an enhanced opportunity for sperm competition in the presence of multiple mating (Olsson *et al.* 1994; Ross 2001).

Multiple paternity can provide an indirect benefit of increasing genetic variation among offspring within a clutch, but direct reproductive advantages (e.g., increased hatching success) of multiple paternity remain unclear (Roques *et al.* 2006). Hypotheses for the advantage of multiple paternity in turtles include the ability to fertilize large clutches and the assurance of male fertility (Olsson *et al.* 1996). However, hatching success generally does not differ between singly and multiply sired clutches (Pearse *et al.* 2002; but see McTaggert 2000; Moon *et al.* 2006). Total egg production in *Chrysemys picta* is larger in clutches with multiple paternity, suggesting that larger females are preferred as mates because they produce larger clutches (Pearse *et al.* 2002). In *Gopherus polyphemus* smaller females lay more clutches with multiple paternity (Moon *et al.* 2006) suggesting that multiple mating may not be beneficial to females, but instead results from females giving in to male harassment (Lee & Hays 2004). Inconsistencies indicate that more studies are needed to broaden our understanding of the direct reproductive advantages of multiple paternity.

The density and sex ratio of turtles at breeding sites may affect male competition (Jensen *et al.* 2006). Jensen *et al.* (2006) hypothesized that high population densities with female biased sex ratios could allow for a “mating frenzy” where males easily encounter females and competition for copulation is decreased (Jensen *et al.* 2006). This hypothesis is supported by a positive correlation between

population size and the proportion of clutches with multiple paternity in the genus *Lepidochelys* but may not be universally applicable to all turtle genera (Jensen *et al.* 2006). To our knowledge, the effect of sex ratio on frequency of multiple paternity has not been addressed for any turtle species.

The purpose of our study was to examine the genetic mating system of the diamondback terrapin (*Malaclemys terrapin*; Emydidae). Terrapins live within salt marshes of the eastern and Gulf coasts of the United States (Ernst *et al.* 1994). The terrapin is in decline in many states and as a result, its conservation status ranges from no official listing or game species to species of special concern, threatened, or endangered in different states (Lester 2007). Terrapin nesting ecology and population dynamic studies document nest site fidelity (Auger 1989; Mitro 2003; Roosenburg 1996) and high fidelity to specific creeks or river sections (Gibbons *et al.* 2001; Roosenburg *et al.* 1999). Terrapin mating aggregations occur in the early spring (Seigel 1979) and fall (Estep 2005) and high levels of gene flow were hypothesized to be the result of male and female terrapins moving substantial distances to mating aggregations, but returning to their home ranges after mating (Hauswaldt & Glenn 2005). However, genetic methods indicate that male-biased dispersal occurs (Hart 2005; Chapter 3) and thus it is possible that gene flow in terrapins is not a result of males and females dispersing long distances to mating aggregations.

The objectives of our study were: **(1)** to determine the proportion of clutches with multiple paternity at four nesting locations within Barnegat Bay, NJ, and one nesting location within the Chesapeake Bay, MD, **(2)** to compare female size, clutch

size, egg size, hatchling size, and hatching success between clutches with single and multiple paternity, (**3)** to determine if sperm storage and remating occurs within and between nesting seasons, (**4)** to determine if males and females move to mating aggregations outside of their home ranges and **(5)** to determine if multiple paternity is correlated with population sex ratios.

4.3 Methods

Collection of females and clutches

Sampling techniques and effort varied among sampling locations due to logistical constraints. We sampled clutches from four locations in Barnegat Bay, NJ, and from Poplar Island in Chesapeake Bay, MD, from 2006-2008. In Barnegat Bay we collected 52 naturally laid clutches on Sedge Island. We identified over 90% of the nesting females and collected blood from 16 of them. Clutches were also sampled at Spizzle Creek (N = 30), North Forsythe (N = 27), and Great Bay Boulevard (N = 47) by inducing gravid females that we hand caught on land or trapped in hoop or fyke nets. We used x-radiography with gravid females to determine total clutch size and then induced egg-laying via interperotineal injection with 10-30 IU/kg Oxytocin (Ewert & Legler 1978). We obtained blood samples from these gravid females. We incubated clutches from Spizzle Creek, North Forsythe, and Great Bay in a hatchery. We sampled natural nests on Poplar Island (N = 32) for which the females' identities were unknown.

We protected all nests with predator excluder cages. Mesh size (13 mm) of predator excluder cages prevented hatchlings from escaping. Upon emergence, we recorded hatchling carapace length, width, and height, plastron length, and mass. We marked hatchlings with unique cohort codes on their marginal scutes. We stored tissue clippings from marginal scutes and/or the tail in ethanol at -20^{o}C. We stored blood samples from nesting females on FTA® nucleic acid cards (Whatman). We released adult females after inducing egg laying and released hatchlings at the original site of the nest or the site of the mother's capture. We also obtained blood samples from males, additional females, and juveniles in Barnegat Bay (N = 1,558). No additional blood samples were collected at Poplar Island in the Chesapeake Bay.

DNA extraction and amplification of microsatellites

We followed manufacturer's instructions for disc removal and modified procedures for FTA purification (Whatman). We punched a 1.2 mm disc (Harris Micro-Punch) from each card. To prevent cross contamination between punches, we rinsed the cutting mat with ethanol between each sample. We also punched a disc from an un-used FTA® card (Whatman) between each sample to prevent cross contamination on the micro punch. We placed the disc in a PCR amplification tube and then rinsed it once with 50 µl of 70% ethyl alcohol for 5 min, twice with 50 µl of FTA® purification reagent for 5 min, and twice with 50 µl of TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) for 5 min. We dried the discs for approximately 10 min on a heating block at 50^{o}C. We conducted PCR analysis within 3 hr of disc washing.

We froze tissue or scute samples at -20^{o}C. We rinsed tissue samples twice with 200 μl of 1x Phosphate Buffer Saline Solution (1x, pH 7.4, 11.9mM Phosphates, 137mM Sodium Chloride, and 2.7 mM Potassium Chloride). We extracted genomic DNA with the DNeasy Tissue Kit following the manufacturer's instructions (QIAGEN).

We designed a 6-microsatellite loci protocol for polymerase chain reaction (PCR) and sequencer load multiplexing. We selected loci from the 27 microsatellite loci that were originally developed for the bog turtle (*Glyptemys muhlenbergii*; King & Julian 2004). Hart (2005) screened 16 loci of these loci and chose 12 based on levels of polymorphism and ease of use. Using allele frequencies at each of the 12 loci from terrapins sampled in Cape May, NJ, and Sandy Hook, NJ, (Hart 2005), we selected 6 microsatellite loci (Table 4-1) for this study based on levels of polymorphism, $P(ID)_{sib}$, and $P(ID)_{unbiased}$ (Waits *et al.* 2001). We ran multiplex PCR products on an ABI 3100 capillary sequencer (Applied Biosystems) together with the internal size standard GENESCAN 500 ROX (Applied Biosystems). We conducted fragment analysis using the software Peak Scanner version 1.0 (Applied Biosystems).

The six loci used in this study did not exhibit null alleles, did not deviate from Hardy-Weinberg equilibrium, and did not exhibit linkage disequilibrium. The six loci combined exhibited high parentage exclusion ($P > 0.985$) and low probabilities of identity, even among siblings ($P \leq 1.9 \times 10^{-3}$). We analyzed 188 clutches for the present study. The mean number of eggs per clutch was 11.6 (range 6-19), based on total egg count from x-rays of induced females and of clutches from naturally laid

nests. Mean number of offspring genotyped per clutch was 8.6 (range 1-19). Altogether, we genotyped 3,166 individuals, including 1,558 adults and 1,608 hatchlings.

Paternity analysis

We determined the frequency of multiple paternity by analyzing clutches in which we could successfully genotype at least 3 hatchlings/embryos. We used GERUD 2.0 to determine the minimum number of fathers in a clutch using a multilocus approach to determine the minimum number of fathers necessary to explain the progeny array (Jones 2005). If there were multiple possible solutions, each solution was assigned a probability score, based on Mendelian segregation (Jones 2005). GERUD 2.0 also provides estimates of the number of offspring sired by each father. GERUD 2.0 can perform with or without the maternal genotype. Since GERUD 2.0 cannot identify mutations, we carefully inspected the results for each clutch to determine if a mutation at a single locus resulted in an additional mother or father. Because the probability of simultaneous mutations at independent loci is extremely low, multiple paternity was rejected only when there was no support from multiple loci. A chi-square test for goodness of fit tested the null hypothesis that fathers contributed equally to multiply sired clutches.

It is important to note that the lack of a known maternal genotype has almost no effect on the ability of GERUD 2.0 to determine the correct number of sires in large samples (>20 offspring) with multiple loci (2-4) genotyped (Jones 2005). To

determine if the lack of a known maternal genotype had an effect on our ability to detect multiple paternity in our smaller samples (<20) we utilized two approaches. First, we simulated progeny arrays based on population allele frequencies in GERUDsim2.0 (Jones 2005) to determine the proportion of progeny arrays in which the minimum number of fathers based on the multi-locus data was the same as the actual number of fathers used to create the progeny array. We determined the effect of the following: knowledge of maternal contribution, number of offspring analyzed, and number of loci analyzed. In each simulation (1,000 iterations), we assumed that progeny arrays had two fathers with equal contributions. Second, to determine the effect that maternal genotype had on actual progeny arrays, we analyzed clutches from Spizzle Creek, North Forsythe, and Great Bay (in which all the maternal genotypes were known) with and without the maternal genotype in GERUD 2.0.

We used a generalized linear mixed model (GLMM) to determine whether the presence of multiple paternity could be predicted by fixed effects such as female carapace length, female mass, total clutch size, mean egg mass, mean egg width, mean hatchling carapace length, mean hatchling mass, and hatching success. We also formally added the number of hatchlings genotyped per clutch in the GLMM to determine whether the number of hatchlings genotyped per clutch influenced our ability to detect multiple paternity. Data on mean female carapace length, female mass, and mean egg width were not available from Poplar Island nests. Data on mean hatchling carapace length, mean hatchling mass, and hatching success from Sedge Island were removed from the analysis because clutches were placed in experimental

treatment plots that significantly altered success of nests (Wnek, 2010). In our GLMM we used both random and fixed effects to determine their affect on the responsible variable. The random component arose because there was repeated sampling within and across years of some female's clutches. Year and female identity were therefore fitted as random effects. We used a logit link function because the response variable was binary (single paternity vs. multiple paternity). The significance of the explanatory terms (i.e. the fixed effects) was assessed by their Wald statistics for each term. Analysis was performed in R version 2.11.0 (R Development Core Team 2010) using the lme4 package with restricted maximum likelihood estimation of variance components (Bates & Maechler 2010).

We used the multi-locus paternity genotypes from the GERUD 2.0 analysis to search for multi-locus genotype matches among all captured males genotyped in Barnegat Bay. Matches were determined using the multi-locus match function of GENALEX 6 (Peakall & Smouse 2006). This function automates the detection of repeated genotypes within a dataset. In cases where more than one multi-locus paternal solution was probable in GERUD 2.0, we attempted to find matches for the paternal solutions with the highest probability scores (≤10 paternal solutions). When a match was found we calculated the multi-locus genotype probability, the probability of a random match to a given specific genotype in GENALEX 6 (Peakall & Smouse 2006).

Although we attempted to sample the majority of hatchlings in each clutch, retained eggs from induced females, undeveloped eggs, and poor sample quality of

deceased embryos lowered our ability to fully sample all clutches. Any incomplete sample can potentially reduce the power to detect multiple paternity compared to complete sampling (Pearse *et al.* 2002). We corrected estimates of the overall frequency of multiple paternity by using subsets of the assayed clutches, grouped according to how many hatchlings were sampled. This method provided an empirical evaluation of the proportion of multiply sired clutches as a function of sample size.

We compared the frequency of multiple paternity using GERUD 2.0 to the frequency of multiple paternity (f_{mm}) for all nesting locations using the computer program FMM (Neff *et al.* 2002). We used two computer programs to compare the frequency of multiple paternity based on the recommendations of Jones *et al.* (2010). The program FMM is based on Bayesian statistics and uses a single-sex model that incorporates information on the (i) number of loci, (ii) number of alleles and their frequencies, (iii) number of clutches, (iv) number of offspring analyzed from each nest, (v) number of fathers and the reproductive skew among them, and (vi) prior probability of multiple paternity (Neff *et al.* 2002). We calculated the mean and 95% confidence intervals of f_{mm} for all locations using seven different combinations of the number of fathers and reproductive skew: two fathers contributing equally (50:50) and skewed (90:10 and 95:5); three fathers contributing equally (33.3:33.3:33.3) and skewed (60:30:10, 50:40:10, and 50:45:5). We assumed a uniform distribution for the probability distribution of multiple paternity (Neff *et al.* 2002; Neff *et al.* 2001).

Multiple paternity and sex ratio

To test whether the frequency of multiple paternity was correlated with sex ratio, we determined the sex ratio of each of the populations, except Sedge Island because capture methods were 100% biased towards nesting females. We determined the sex ratio at Spizzle Creek by counts of males and females captured in hoop traps and on land from June-September (2006-2007). We determined the sex of all captured individuals on the basis of carapace length, tail thickness, and cloacal positioning (Tucker *et al.* 2001). We determined sex ratio at North Forsythe by counts of males and females captured in hoop traps, fyke nets, and on land from June-September (2006-2008). We did not directly estimate sex ratio at Great Bay, but a mark-recapture study in 2001 gave a female sex bias of 2:1 (Avissar 2006). This ratio was a decrease from a female biased sex ratio (~ 4:1) measured 13 years earlier (Rountree *et al.* 1992) before the construction of a paved road adjacent to the creek in the early 1990s. Approximately, 10-50% of the nesting females crossing the road are killed by vehicles (Hoden & Able 2003; Szerlag & McRobert 2006). Given that our sampling of clutches occurred 8 years since the last survey and no measures have been taken to prevent female road mortality, we estimated that the sex ratio is now close to 1:1. Finally, we determined sex ratio within the Poplar Island archipelago by counts of males and females captured in fyke nets, in crab pots, and on land from May-August 2009.

We searched the literature for turtle populations in which both the frequency of multiple paternity and sex ratios were available. We recorded the species, study

location, number of clutches sampled, frequency of multiple paternity, number of males and females captured, sampling methods, and years sampled. We attempted to determine sex ratios within the year(s) that clutches were sampled for multiple paternity, but in some cases these data were not available so we utilized data from different years and assumed that the sex ratio did not change over time. We excluded data from studies in which collections were completely biased towards one sex, such as road patrols for aquatic turtles during the nesting season. Although, multiple paternity has been studied in a number of sea turtle species and populations, we excluded these data because accurate estimates of adult sex ratios are difficult to obtain (Lovich 1996).

4.4 Results

The frequency of multiple paternity differed among sampling sites and ranged from 12.5 to 45.7% (Table 4-2). We rejected the null hypothesis that the observed frequency of multiple paternity was independent of sampling site ($\chi^2 = 12.08$, df = 4, P = 0.017; Table 4-2). When using model-based population estimates of f_{mm}, which estimates the frequency of multiple paternity assuming different paternity skews, the frequency of multiple paternity continued to differ among sampling sites (Table 4-2). This suggested that even if all clutches had high paternity skews our sample sizes were large enough to find differences in the frequency of multiple paternity between sites. Using post-hoc chi-square analysis ($\alpha = 0.04$ based on Bonferroni correction), we found that the difference in the observed frequency of multiple paternity was

driven by differences between Sedge Island and Great Bay ($\chi^2 = 5.52$, P = 0.02) and Sedge Island and Poplar Island ($\chi^2 = 9.55$, P = 0.002).

Simulations in GERUDsim2.0 indicated no effect of maternal sampling on our ability to infer multiple paternity (Figure 4-1). Due to computational limitations, we could not analyze the effect when genotyping >4 loci. We can assume a power of 1 when 6 loci and ≥6 offspring were genotyped and a power >0.8 when 6 loci and >4 offspring were genotyped (regardless of maternal sampling) because the proportion of runs inferring correct number of sires approaches 1 when 3 loci and ≥6 offspring were genotyped (Figure 4-1). We also found no effect on sampling the maternal genotype in actual progeny arrays from Spizzle Creek, North Forsythe, and Great Bay. All nests in which we determined multiple paternity when making use of the known maternal genotype, also indicated multiple paternity when we excluded the maternal genotype from the analysis. Thus, the frequency of multiple paternity at Spizzle Creek, North Forsythe, and Great Bay did not change between analyses (with and without the maternal genotype) and we can confidently assume that the observed frequency of multiple paternity at Poplar Island and Sedge Island were not biased because of a lack of known maternal genotypes.

We genotyped at least 3 hatchlings from 174 of the 188 clutches analyzed. Overall frequency of multiple paternity was 28.7% (50 of 174 clutches; Table 4-3). When we limited the analyses to clutches in which we sampled at least 6 hatchlings the frequency of multiple paternity was 27.9% and when we limited the analyses to clutches in which we sampled at least 9 hatchlings the frequency of multiple paternity

was 28.4% (Table 4-3). Limiting the analysis to clutches sampled more extensively did not affect the estimated frequency of multiple paternity ($\chi^2 = 0.06$, P = 0.97). The smallest mean value of the model-based population estimate of f_{mm} (Table 4-2) was 16.7% (95% CI: 12-28%), assuming 3 fathers contributed equally to the clutches. The maximum mean value of the model-based population estimate of f_{mm} (Table 4-2) was 66.9% (45-88%), assuming that two fathers had a reproductive skew of 95% and 5%. For clutches with double paternity (N = 41), fathers contributed equally in 34 of the clutches and skewed in 7 of the clutches. For clutches with triple paternity (N = 9), fathers contributed equally in 7 of the clutches and skewed in 2 of the clutches. Paternal contributions departed significantly from equality (χ^2 test for goodness of fit P < 0.05) in 17.1% of the cases of double paternity and in 22.2% of the cases of triple paternity. Overall, only 18% of the clutches with more than one father differed from equality, suggesting that the true frequency of multiple paternity was likely closest to the model-based population estimate of f_{mm} where we assumed fathers contributed equally to clutches (i.e. 50:50 and 33:33:33).

When an extra allele in a clutch occurred at a single locus, we considered it to be the result of *de novo* mutation, rather than an additional father. A total of 29 scored alleles indicated mutation events. Eight of these arose in the paternal germ line, 16 in the maternal germ line, and 5 were ambiguous. Twenty-nine mutations in ca. 19,296 allelic transmissions (1,608 hatchlings X 2 alleles X 6 loci) was equivalent to 1 mutation for every 665 meiotic events, which was a typical rate (1.5×10^{-3}) reported for microsatellite loci (Ellegren 2000).

We were able to match 7 reconstructed paternal genotypes to male genotypes among 142 clutches assayed from Barnegat Bay. The expected frequencies of the 7 genotypes in the population (based on Hardy-Weinberg equilibrium) were sufficiently low enough to indicate that the true sire had actually been identified (Table 4-4). All identified fathers and their clutches originated from North Forsythe, the most intensively sampled site. The mean distance between the location where the gravid female and the sire of her clutch were captured was 224.2 m (range: 0 -724.2; Table 4-4). Although, captured locations of gravid females and sires did not exclusively indicate the location of the mating event, the locations did indicate that both females and sires were utilizing the same area as part of their home range.

Data from multiple clutches within a season or between seasons indicated the use of stored sperm both within seasons and between seasons or remating with the same individual. Analyses of paternal alleles in successive clutches of females within a season (N = 3) indicated that the same male's sperm fertilized these successive clutches. We analyzed two successive clutches from one female in which one father sired both clutches, three successive clutches from one female in which one father sired all three clutches, and two successive clutches from one female in which three fathers sired both clutches. In the last example, the dominant father changed between clutches (1:1:7 and 1:7:3; sire 1, 2, and 3 respectively in the first and second clutch). Analyses of paternal alleles in successive clutches of females between nesting seasons (N = 2) indicated that the same male's sperm fertilized these successive clutches. Paternal alleles from both females' clutches in 2007 and 2008 indicated that

the second year clutches were fertilized by the first year male. None of the successive clutches, either within or between seasons, were sired by additional fathers (i.e. remating between clutches). Therefore, we were unable to compare whether stored sperm or sperm from a recent mating event differentially affected hatching success, hatchling mass, or hatchling size. Finally, no paternal genotypes were found in clutches from more than one female among the 142 clutches assayed in Barnegat Bay and the 32 clutches assayed in the Chesapeake Bay.

Our generalized linear mixed model (GLMM) indicated that the presence of multiple paternity could be significantly predicted by any of the tested fixed effect (female carapace length, female mass, total clutch size, mean egg mass, mean egg width, mean hatchling carapace length, mean hatchling mass, and hatching success). Furthermore, the number of hatchlings genotyped in each clutch did not significantly influence our ability to detect multiple paternity (Table 4-5).

Sex ratio varied greatly among trapping locations. In Barnegat Bay, Spizzle Creek had female biased sex ratio of 1.4:1 based on trapping data from 2006-2007 (Table 4-6). North Forsythe had a female biased sex ratio of 1.6:1 from 2006-2008 (Table 4-6). In Chesapeake Bay, Poplar Island had a female biased sex ratio of 9:1 in 2009 (Table 4-6). Within the *M. terrapin* populations we sampled, a polynomial relationship between sex ratio and frequency of multiple paternity ($r^2 = 0.99$, $F_{2,2} = 119.9$, $P = 0.008$, Figure 4-2) suggested that multiple paternity decreased as sex ratio bias in either direction increased. Furthermore, when we included data available from other turtle species (Table 4-6), a polynomial regression continued to explain the

relationship between sex ratio and frequency of multiple paternity ($r^2 = 0.88$, $F_{2,9} = 32.5$ $P < 0.0001$, Figure 4-2). Although we initially excluded two populations of captive raised turtles from the analysis, inclusion did not alter the relationship ($r^2 = 0.76$, $F_{2,11} = 17.5$, $P < 0.001$, Figure 4-2). If the sex ratio of at Poplar Island is considered to be an outlier data point, then the relationship between sex ratio and frequency of multiple paternity remains significant, but is a positive linear fit rather than polynomial ($r^2 = 0.688$, $F_{1,11} = 24.3$, $P < 0.001$). This relationship suggests that multiple paternity increases as the female biased sex ratio increases.

4.5 Discussion

The frequencies of multiple paternity differed between sampling sites, ranging from 12.5 to 45.7% (Table 4-2). Overall frequency of multiple paternity in our study was 28.7%. The majority of clutches in which we detected multiple paternity had 2 fathers with no paternity skew (68% of 50 clutches). This suggested that the true frequency of multiple paternity was probably closer to statistical estimates based on 2 fathers with 50:50 paternity skew (Table 4-2). Our occurrence of multiple paternity (9.5 to 48.8 %, Table 4-2, 50:50 paternity skew) was within the range reported in 12 studies of 9 other turtle species [*Caretta caretta* (Bollmer *et al.* 1999; Harry & Briscoe 1988; Moore & Ball 2002), *Chelonia mydas* (Parker *et al.* 1996), *Chrysemys picta* (Pearse *et al.* 2002), *Dermochelys coriacea* (Crim *et al.* 2002), *Emys orbicularis* (Roques *et al.* 2006), *Gopherus polyphemus* (Moon *et al.* 2006), *Lepidochelys olivacae* (Hoekert *et al.* 2002; Jensen *et al.* 2006), *Testudo graeca* (Roques *et al.*

2004), *Testudo horsfieldii* (Johnston *et al.* 2006)]. Despite the moderate occurrence of multiple paternity, we did not find that any difference between clutches with multiple vs. single paternity in female size, clutch sizes, hatching success, egg size, or offspring size, suggesting that multiple paternity did not provide direct immediate benefits to offspring.

Three females either utilized stored sperm for consecutive clutches within nesting seasons or mated with the same male more than once. Two females showed the same pattern between consecutive nesting seasons. Sperm storage is a more likely explanation than multiple matings with the same male. In *M. terrapin*, male spermatogenesis peaks in the fall and spermatozoa can be found within the epididymides until late spring (Lee 2003). Female testosterone levels peak in April and females ovulate follicles and produce multiple clutches throughout the summer (Lee 2003). This suggests that mating occurs in the spring and possibly in the fall (Lee 2003). Asynchronous reproductive cycles of males and females support the use of stored sperm within a nesting season, but do not explain why some females utilized stored sperm rather than remating between consecutive nesting seasons. Both females were from Sedge Island, where the frequency of multiple paternity was the highest. Although we do not have sex ratio data from this location, our polynomial regression (Figure 4-2) suggests a female biased sex ratio of 3.1:1 at this location. Usage of stored sperm over consecutive nesting seasons may be common because females may not always encounter a male during the mating season.

In one of the females with two consecutive clutches within a nesting season, the same three fathers sired hatchlings from both clutches but the dominant father changed between clutches (7:1:1 and 3:7:1; sire 1,2, and 3 respectively in the first and second clutch). This suggested that the female utilized stored sperm. Paternity skew also changed between consecutive clutches in a season in Oyster Bay, New York terrapin clutches (Hauswaldt 2004). A female laid two clutches in a single nesting season with paternity skew ratios of 2:5:0 and 2:0:9 (Hauswaldt 2004). Interestingly, despite the usage of stored sperm of sire #1 in both clutches, sire #2 was not implicated as the father in any hatchlings in clutch #2. If indeed sperm was stored from sire #2, but not utilized, it is possible that the use of stored sperm changes as a function of the number of eggs produced in each oviduct or the location in which the sperm is stored in the oviducts. Another possibility is that female turtles have the ability to innately select whether sperm from particular males is stored in the oviduct.

The current study documented a mean distance between mating pairs of 224.2 m (range: 0 -724.2 m; Table 4-5). The data suggest that males and females do not move significant distances to mating aggregations, rather mating aggregations occur within their home range. Thus, the hypothesized long distance movement of males and females to mating aggregations (Hauswaldt & Glenn 2005) likely does not occur at our sites and thus may not be responsible for the high levels of gene flow documented in this species.

Female biased sex ratios increased the frequency of multiple paternity in freshwater, estuarine, and terrestrial turtle species (Table 4-6, Figure 4-2).

Furthermore, we found a maximum frequency of multiple paternity at a female biased sex ratio of 4.7:1 (inflection point). We propose that the initial increased frequency of multiple paternity (from male bias to female bias) could be caused by decreased male competition and increased frequency of multi-male sperm storage by females. When sex ratios are male-biased or equal, male competition may be high due to limited numbers of females (Emlen & Oring 1977). In *M. terrapin*, male competition may occur in mating aggregations where single males are often observed next to mounted pairs (Estep 2005). When sex ratios are male-biased, females may select a single mate of the highest quality. In addition, since females can store sperm, it allows them the choice of not remating within or between reproductive seasons if they have mated with a high quality male (Pearse & Avise 2001). When sex ratios change from male biased or equal to female biased, direct male competition and/or sexual selection is relaxed (Emlen & Oring 1977). Because of the reduction in sexual selection, males can easily encounter females and females are less likely to reject mating attempts. In populations with female biased sex ratios, females may use sperm storage more frequently because the chances of mating with another male might be low. Sperm storage may also be more common because it allows for sperm competition to occur and thus indirect male competition could occur. Some females that store sperm within and between years may in fact encounter males again, and thus polyandry could be elevated in female biased populations as a result of temporal polyandry and sperm storage. However, as female biased sex ratios become even larger (>4.7:1), females encounter males less frequently. Males in mating aggregations may quickly become

depleted of sperm stored in the epididymides, reducing the possibility of multiple paternity. It is possible that by the time the female encounters another male for mating in future years, stored sperm within her oviduct is less robust. Other studies suggest that the quality of stored sperm decreases through subsequent reproductive seasons and as a result hatching success, hatchling mass, and hatchling size are reduced (Roques *et al.* 2006). Thus, when females mate again, last male precedence takes place because the newest sperm outcompetes the oldest sperm, leading to lower levels of multiple paternity in clutches, regardless of multiple mating in the lifetime of the female.

Conclusions and Conservation Implications

In the current study, genetic parentage analyses on several populations of nesting diamondback terrapins in Barnegat Bay and in the Chesapeake Bay indicated that, (1) the frequency of multiple paternity was significantly different between nesting locations, (2) overall frequency of multiple paternity is 12.5 to 45.7%, (3) sperm storage occurs both within and between nesting seasons and with sperm from single and multiple males, (4) changes in paternity skew between clutches from a single female may be the result of changes in the use of stored sperm, (5) males and females mate with individuals found within their home ranges, (6) the frequency of multiple paternity was not correlated with female size, clutch size, egg size, hatchling size, or hatching success, (7) a non-linear increase in the frequency of multiple paternity is correlated with changes from male biased sex ratios to female biased sex

ratios, with the maximum frequency of multiple paternity occurring at a female biased sex ratio of 4.7:1.

Understanding the mating system of species is particularly important for small or declining populations (Anthony & Blumstein 2000). Multiple paternity typically causes increases in the effective population size and decreases the variance in effective population size per generation and thus significantly improves the maintenance of genetic diversity in a conservation context (Fiumera *et al.* 2004). When populations are extremely small the effects of within clutch multiple paternity and sperm storage (females could utilize sperm from males no longer in the population) on increasing the effective population size has a much greater influence on the maintenance of genetic diversity than when populations contain thousands of males and females in a mating system with temporal (e.g., between years) polyandry (Pearse & Anderson 2009).

The diamondback terrapin has undergone significant declines in population size throughout its entire range in the past due to overharvesting (Conant 1964) and some populations are again experiencing declines (Seigel & Gibbons 1995; Roosenburg *et al.* 1997). Current declines in population size are due to a variety of human induced threats (reviewed in Seigel & Gibbons 1995). Some threats that differentially affect males and females include boat strikes (Roosenburg 1990), vehicle strikes (Wood & Herlands 1997), and commercial style crab pots deaths (Bishop 1983; Roosenburg *et al.* 1997). Boat strikes (Cecala *et al.* 2008) and vehicles strikes (Wood & Herlands 1997) primarily affect adult females while males are

primarily affected by drowning in crab pots (Roosenburg *et al.* 1997). Our data demonstrate that highly biased female populations (>4.7: 1 females: males) and male-biased populations reduce the frequency of multiple paternity within clutches. In addition to reduced multiple paternity within clutches, temporal multiple paternity likely occurs at a lower rate in extremely female biased populations due to Allee effects caused by low densities of males (Stephens & Sutherland 1999). Thus populations that are declining and are experiencing extremely biased female sex ratios will likely suffer the most from significant declines in effective population size and genetic diversity over time. Furthermore, diamondback terrapins exhibit a pattern of temperature dependent sex determination (TSD) in which high incubation temperatures produce females and low incubation temperatures produce males (Jeyasuria *et al.* 1994). Species with this pattern of TSD are assumed to be highly sensitive to global climate change (Walther *et al.* 2002) because rapid changes in environmental conditions might lead to large female biases in offspring sex ratio (Hulin *et al.* 2009). Management and recovery plans for populations experiencing population declines leading to female-biased sex ratios should focus on removing the threats causing population decline and should consider utilizing nest protection, head-starting, and breeding programs to reduce variability in reproductive success, increase the population size, and reduce the extremely biased female sex ratio of the population.

Table 4-1. Characteristics of the 6-microsatellite multiplex kit.

Loading and PCR Plex	Locus	GenBank Accession #	Primer Concentration	Size Range (bp)	Label
A	*Gmu* B08	AF517229	0.2	211-241	6-FAM
A	*Gmu* D121	AF517252	0.2	124-188	NED
A	*Gmu* D62	AF517241	0.25	127-175	HEX
B	*Gmu* D87	AF517244	0.2	224-276	6-FAM
B	*Gmu* D114	AF517251	0.2	88-124	NED
B	*Gmu* D90	AF517247	0.25	111-147	HEX

PCR chemistry: 20 µl PCR reactions using 5-15 ng of DNA or 1.2 mm Whatman blood punch, 0.3175 mM dNTPs, 1x GoTaq Flexi Buffer (Promega), 3.75 mM $MgCl_2$, 0.2-0.25 mM primer, 0.5 units of GoTaq polymerase (Promega).

PCR thermocycling: 94°C for 2 min, 35 cycles of denaturation at 94°C for 45 s, annealing at 56°C for 45 s, extension at 72°C for 2 min. Final extension at 72°C for 10 min.

Table 4-2. Summary of the observed frequency of multiple paternity and the estimated population frequency of multiple paternity with 95% confidence intervals (in brackets) for the diamondback terrapin from: Sedge Island (SI), Spizzle Creek (SC), Lighthouse Center (LHC), North Forsythe (NF), Great Bay Boulevard (GB), and Poplar Island (PI), and all locations combined. Seven scenarios were considered for the number of fathers and their reproductive skews (in parentheses) to estimate population frequencies. See text for model and simulation details. Results of chi-square test of independence between the five sites frequency of multiple paternity are given.

Sampling Site	Observed	Estimated						
		2 (50:50)	2 (90:10)	2 (95:5)	3 (33:33:33)	3 (60:30:10)	3 (50:40:10)	3 (50:45:5)
SI (N = 46)	45.7	48.8 [34-62]	66.0 [46-91]	85.0 [50-97]	39.6 [26-52]	46.9 [30-65]	44.3 [29-59]	46.6 [33-61]
SC (N = 28)	25.0	27.8 [8-47]	46.5 [19-74]	62.1 [28-99]	25.0 [10-44]	26.7 [20-47]	30.9 [11-47]	28.1 [12-48]
NF (N = 23)	34.8	31.4 [10-53]	48.1 [20-83]	62.7 [26-98]	24.4 [3-47]	30.3 [12-53]	25.9 [9-50]	29.5 [11-54]
GB (N = 45)	22.2	19.0 [8-34]	31.8 [13-55]	47.0 [19-82]	15.8 [4-28]	21.6 [6-35]	18.5 [8-29]	21.2 [7-32]
PI (N = 32)	12.5	9.5 [0-24]	18.6 [4-38]	26.1 [5-63]	5.81 [0-16]	6.7 [0-21]	6.7 [0-20]	7.8 [1-20]
All Locations (N = 174)	28.7	26.5 [21-38]	42.2 [32-64]	66.9 [45-88]	16.7 [12-28]	30.8 [20-33]	23.0 [21-26]	27.9 [22-34]
Chi-square p-value	0.017	0.001	< 0.001	< 0.001	0.009	0.002	0.003	0.004

Table 4-3. Frequencies of multiple paternity in groups of diamondback terrapin clutches differing in the number of hatchlings sampled.

Hatchlings sampled per clutch (average)	Number of clutches	Number of detected multiply sired clutches	Observed frequency of multiply sired clutches
≥ 3 (9.09)	174	50	28.7
≥ 6 (9.68)	154	43	27.9
≥ 9 (10.77)	109	31	28.4

Table 4-4. Expected frequencies for the seven multilocus genotype matches between the deduced sire of a clutch and an adult male that was physically captured. Dates that the adult males and gravid females were captured are given. The distance (m) between the adult male and gravid female are given. Recaptured males were within <50m of the first capture location.

Clutch ID	Father ID	Mother ID	Capture Date(s) of Male	Capture Date of Gravid Female	Distance (meters)	Expected paternal genotype frequency
Clutch 2	HIJX	CJPQ	7/21/2006	6/5/2007	37.3	6.5E-09
Clutch 4	BIJV	BHIP	8/9/2006; 6/20/2007	6/18/2007	146.7; 143.7	2.1E-08
Clutch 6	ABJK	CIJP	6/30/2007	6/21/2007	7.2	4.7E-11
Clutch 8	PVWX	ACHO	7/6/2006; 7/14/2006	6/21/2007	11.4; 49.0	2.3E-10
Clutch 9	BHIV	IJOPV	8/9/2006; 6/21/2008	6/24/2007	717.5; 724.2	3.1E-10
Clutch 13	BJNV	HJOQV	8/11/2006	6/17/2008	628.9	8.8E-10
Clutch 16	AHKMU	AHINV	6/21/2008	6/21/2008	0	2.5E-09

Table 4-5. Generalized linear mixed model of multiple paternity vs. single paternity in the diamondback terrapin. Parameter estimates of clutch size, female size, egg size, hatching size, hatching success, and number of hatchlings genotyped are given.

	Estimate	Standard Error	Wald Statistic	P-value
Total clutch size	0.14	0.18	0.76	0.45
Female CL (mm)	0.07	0.06	1.15	0.25
Female mass (g)	0.00	0.00	-1.08	0.28
Egg width (mm)	0.34	0.42	0.82	0.41
Egg mass (g)	-0.23	0.85	-0.28	0.78
Hatchling mass (g)	1.86	2.38	0.78	0.43
Hatchling CL (mm)	-0.17	0.13	-1.25	0.21
Hatching success	0.52	0.53	0.99	0.32
# of Hatchling genotyped	-0.75	1.16	-0.64	0.52

Table 4-6. Summary of studies in which the frequency of multiple paternity (% MP) and sex ratio data are available. We excluded studies of marine turtles because accurate estimates of adult sex ratios are difficult to obtain. An * indicates a captive population. A ^ indicates see text for details on sex ratio estimation.

Species	Location	# nests	% MP	Author	Sex Ratio (F:M)	Author
C. serpentina	E. S. George Reserve, MI	40	42	Mcguire unpublished	2.63	Congdon et al. 1986
C. picta	South Potter's Marsh, IL	215	10.7	Pearse et al. 2002	0.67	Pearse et al. 2002
C. picta	E. S. George Reserve, MI	80	16.3	Mcguire unpublished	0.43	Congdon et al. 2003
C. insculpta	Algonquin Park, ON	10	50	Galbraith 1991	4.11	Quinn and Tate 1991
E. orbicularis	Donana National Park, ES	20	10	Roques et al. 2006	1.04	Roques et al. 2006
G. agassizii	Las Vegas, NE*	12	42	Palmer et al. 1998	1.50	Palmer et al. 1998
G. polyphemus	Hillsborough County, FL	7	28.6	Moon et al. 2006	1.28	Mushinsky et al. 1994
T. graeca	Donana National Park, ES	15	20	Roques et al. 2004	0.69	Diaz-Paniagua et al. 2001
T. horsfieldii	MN*	11	27	Johnston et al. 2006	0.67	Johnston et al. 2006
M. terrapin	Poplar Island, MD	32	9.5	this study	9	this study
M. terrapin	Spizzle Creek, NJ	28	27.8	this study	1.47	this study
M. terrapin	North Forsythe, NJ	23	31.4	this study	1.64	this study
M. terrapin	Great Bay Blvd, NJ	45	19.0	this study	1	Avissar 2006, Rountree et al. 1992^
M. terrapin	Oyster Bay, NY	23	21	Hauswaldt 2004	1	Hauswaldt 2004

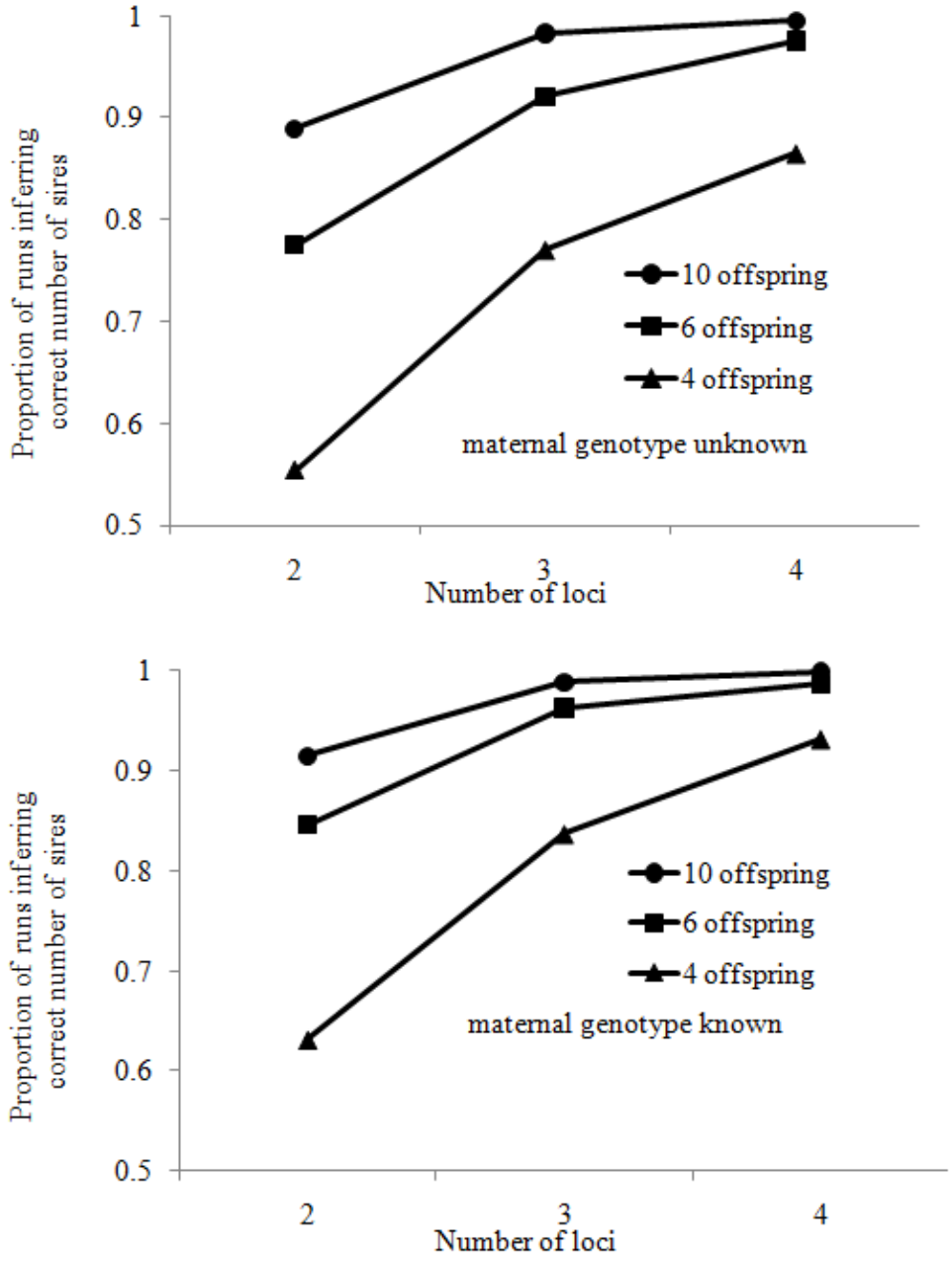


Figure 4-1. Probabilities of success when GERUD2.0 is used to reconstruct the number of sires from a progeny array with one mother whose genotype is either unknown (top panel) or known (bottom panel). Each data point is based on 1,000 simulation runs in Gerudsim2.0, assuming two fathers with equal paternity contributions. Note that the results when the mother's genotype is known (bottom panel) do not differ substantially from the results when the maternal genotype is unknown (top panel), especially when >3 loci are genotyped in six or more offspring.

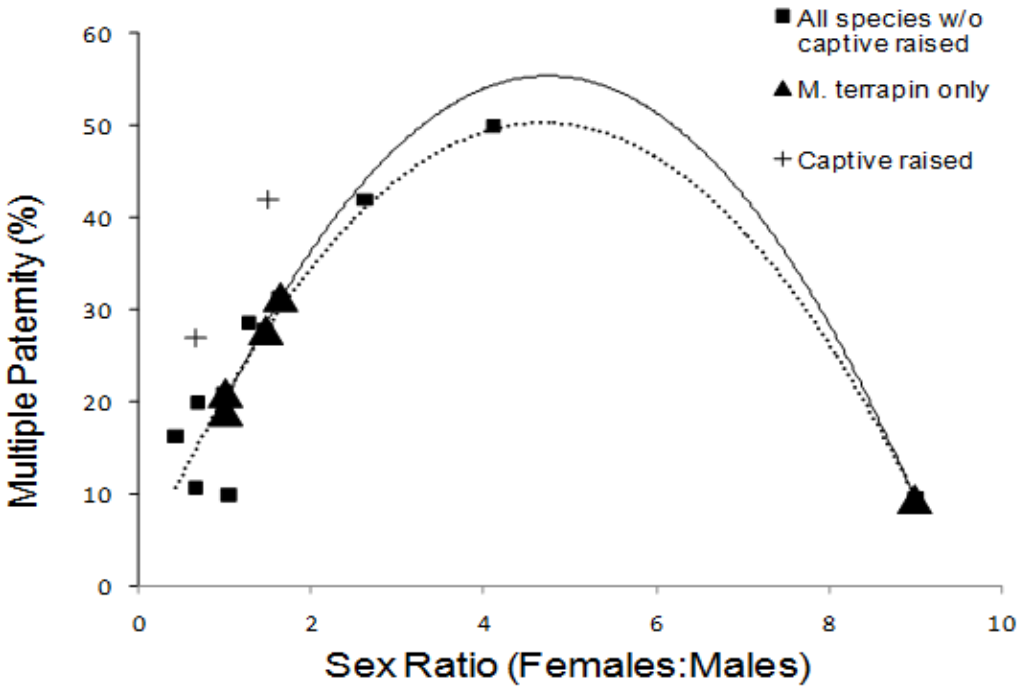


Figure 4-2. Multiple paternity (%) plotted against the estimated sex ratio for all studies shown in Table 6. The solid line indicates the polynomial regression for *M. terrapin* only ($y = -2.5325x^2 + 24.02x - 1.5488$, $r^2 = 0.99$, $p = 0.008$). The dashed line indicates the polynomial regression for all species listed in Table 6, excluding those that were captively raised and bred ($y = -2.1898x^2 + 20.534x + 2.212$, $r^2 = 0.88$, $p < 0.001$). The polynomial regression of all species listed in Table 6, including those that were captively raised was also significant ($y = -2.1465x^2 + 19.808x + 5.1095$, $r^2 = 0.76$, $p = 0.0004$).

CHAPTER 5: Constraints on egg size, optimal egg size theory, and latitudinal reproductive variation in the diamondback terrapin (*Malaclemys terrapin*)

5.1 Abstract

Optimal egg size (OES) theory predicts that in a given environment females will divide investment per offspring to produce offspring of an optimal size that will maximize maternal fitness. However, egg size is often correlated with female body size. Furthermore, egg size and clutch size often vary with latitude. Morphological constraints, such as pelvic aperture width, on egg size have been hypothesized to account for the correlation between egg size and female body size. We use data from several nesting populations of the diamondback terrapin, *Malaclemys terrapin*, in Barnegat Bay, New Jersey to evaluate optimal egg size theory and morphological constraints on egg size in *M. terrapin*. We also evaluate the latitudinal variation in female body size and in clutch characteristics using data from this study and other previously published investigations. We find a correlation between female body size and egg size suggesting a constraint on egg size in *M. terrapin*. However, pelvic aperture width does not increase at the same rate as egg width on female body size, suggesting that pelvic aperture width is not the proximate cause of egg width constraint. Furthermore, we find a trade-off between clutch size and egg size in *M. terrapin* supporting OES theory, but our data also supports optimization of clutch size rather than egg size in *M. terrapin* of Barnegat Bay, NJ. Latitudinal variation in clutch size and egg size suggest that egg size is optimized in southern latitudes, while clutch size is optimized in northern latitudes.

5.2 Introduction

Optimal egg size theory (OES) predicts that in a given environment females will divide the energy available for reproduction into eggs of an optimal size (Smith & Fretwell 1974). It is generally assumed that larger eggs contain more energy that can be used to produce larger or better provisioned offspring (Long & Rose 1989; Wilkinson & Gibbons 2005) and that a female's total reproductive output is limited by resources (Smith & Fretwell 1974) or maternal reproductive capacity (Wilkinson & Gibbons 2005). Thus, OES predicts that females will produce the largest eggs possible until further increases in egg size compromise the female's fitness by reducing the number of offspring (Brockelmam 1975; Smith & Fretwell 1974). In OES, variation in reproductive output in a population should result primarily in variation of the number of offspring produced and secondarily in variation of offspring size (Brockelmam 1975; Smith & Fretwell 1974).

Some research studies support the OES theory (Einum & Fleming 2000), however, others document variation in investment per offspring among females and within populations (Hendry *et al.* 2001; McGinley *et al.* 1987; Roosenburg & Dunham 1997). Variation in investment per offspring is often correlated with maternal body size (reviewed in Roff 1992). Because the OES predicts that investment per offspring should be relatively conserved among individuals in a population and that the variation in energy available for reproduction should be expressed primarily in terms of offspring number, other hypotheses have been

suggested to account for the variation in investment per offspring especially in relation to its correlation with maternal body size.

The constraint hypothesis postulates that in some populations egg size might be constrained by certain morphological features of female body size, and that egg size increases with female body size until females are large enough to produce eggs of an optimal size (Congdon & Gibbons 1987; Congdon *et al.* 1983; Ford & Seigel 1989; Long & Rose 1989; Sinervo *et al.* 1992; Sinervo & Licht 1991b). Some reptile species, especially smaller bodied turtles, have egg widths that are constrained by either the pelvic aperture (Congdon & Gibbons 1987; Kratochvil & Frynta 2006) or the caudal gap (Clark *et al.* 2001). Pelvic aperture, a structure that eggs must pass through during oviposition, may be adaptively compromised. Although a larger pelvic aperture might allow for larger, wider eggs it can negatively affect locomotor performance (Congdon & Gibbons 1987). Beyond female morphological features, egg size could also be physiologically constrained by hormonal levels. One study found that elevated testosterone in younger females of *Chrysemys picta* reduced egg size (Bowden *et al.* 2004).

If there are morphological constraints on egg width, females could potentially increase egg mass by increasing egg length rather than width. However, there may be functional constraints on egg elongation (Ji *et al.* 2006; Sinervo & Licht 1991a). One constraint might be that spherical eggs are more resistant to desiccation than elongated eggs due to their lower surface area to volume ratio (Long & Rose 1989). Elongated eggs have reduced packing efficiency when compared to spherical eggs,

and thus increased egg elongation could also potentially limit available space in the oviduct and potentially reduce clutch size (Long & Rose 1989).

Complicating the study of OES theory, which typically is studied in one selective environment, is that many species' ranges extend along a latitudinal gradient. For example, intraspecific latitudinal trends in body size have been found species such as common musk turtles (*Sternotherus odoratus*, Tinkle 1961) and painted turtles (*Chrysemys picta*, Moll 1973). Latitudinal trends in clutch size, such that northern populations lay relatively larger clutches, have also been documented in freshwater turtles (Cagle 1954; Christiansen & Moll 1973; Moll 1973; Powell 1967; Tinkle 1961). Latitudinal trends in clutch size have been explained by the balanced mortality hypothesis (Fretwell 1969). The hypothesis predicts that large clutch sizes have a selective advantage in northern latitudes because they compensate for greater mortality rates in unpredictable environments (Price 1974; Tinkle 1961). In southern latitudes resources may be limited due to increased competition or harsh environmental conditions and selection may favor a decrease in clutch size (Cody 1966) and individuals may produce fewer but more competitively able offspring (Moll & Legler 1971). In contrast to latitudinal trends in clutch size and female body size, no latitudinal trends were found between latitude and body-size adjusted annual clutch mass (which represents total parental investment in a species with no parental care) in a meta-analysis of 146 turtle species (Iverson *et al.* 1993). This suggests that latitude does not affect total parental investment, but rather that increases in clutch size are represented as a tradeoff resulting in decreases in egg size.

The purpose of this study is to evaluate egg size constraints, OES theory, and assess latitudinal trends in reproductive output in the diamondback terrapin, *Malaclemys terrapin.* To evaluate OES theory and the pelvic constraint hypothesis we utilize clutch size data collected as part of a larger study (Chapter 4, Wnek 2010) of *M. terrapin* in Barnegat Bay, NJ. To assess latitudinal trends in reproductive output we utilized literature reported values of female size and clutch size characteristics in *M. terrapin.*

5.3 Methods

Study species

The diamondback terrapin, *M. terrapin,* inhabits the coastal brackish estuaries and marshes along the Atlantic and Gulf coasts of the United States, from Corpus Christi, Texas to Wellfleet, Massachusetts (Iverson 1992). This species is a habitat generalist that utilizes both the terrestrial and aquatic habitat of an estuary for foraging, mating, nesting and hibernation. Many populations are in decline primarily due to loss of habitat, road mortality (Szerlag & McRobert 2006), crab pot mortality (Roosenburg *et al.* 1997; Wood & Herlands 1997), and harvesting for food trade (Roosenburg *et al.* 2008).

In the New Jersey, the nesting season extends from late May/early June to late July (Burger & Montevecchi 1975; Szerlag & McRobert 2007; Wood & Herlands 1997)**.** Females prefer to nest in areas with sandy soils, little vegetation, and higher elevation (Burger & Montevecchi 1975; Butler *et al.* 2004). These areas can be found

on dunes behind barrier islands, on upland marshes, and along roadsides. In New Jersey reported clutches range in size from 4 to 18 eggs (Montevecchi & Burger 1975) and multiple clutches within a reproductive season have been observed (Szerlag & McRobert 2007).

Across their range, terrapins exhibit a large range of adult female body sizes (101-220 mm PL; reviewed in Brennessel 2006). Among North American turtles, adult diamondback terrapins have the greatest size disparity between males and females (Carr 1952). Adult males average about a third the size of females. Sexual dimorphism in body size of the terrapin may have been favored by natural selection to increase clutch size and pelvic aperture width of females. However, different studies have reported different relationship between female size, clutch size, clutch mass, egg size, and pelvic aperture width (Table 5-1); making it difficult ascertain whether terrapins fit the models of OES and egg size constraint. Clutch size is positively correlated with female body size in most terrapin studies (Table 5-1). However, these studies also documented a lack of correlation between female size and any measure of egg size and female pelvic aperture width and egg size (Table 5-1), suggesting that egg size is not constrained in *M. terrapin*. Furthermore, inconsistent with OES, clutch size and egg size tradeoffs have not been documented (Table 5-1), but larger coefficients of variation in clutch size compared to variation in egg size are consistent with OES (Roosenburg & Dunham 1997).

A cline in *M. terrapin* egg mass and length was proposed (Seigel 1980) and later confirmed by others (Table 5-2). A cline in egg width was documented

(Zimmerman 1989) but was later refuted (Table 5-2), suggesting that egg mass was greater in southern populations due to increases in egg length, but not egg width (Allman 2006). These results point to a possible constraint on egg width in *M. terrapin*. A clinal variation in clutch size was also proposed (Seigel 1980) and later confirmed by others (Table 5-2). However, inconsistent results in tests of a clinal variation in female body size and total clutch mass (Table 5-2) complicate interpretation. Furthermore, most studies included data from only two to five populations and the data were statistically analyzed using ANOVA or t-tests rather than regressions between latitude and female or clutch characteristics.

Field sites

Our field sites were located within Barnegat Bay (39^{o}N) along New Jersey's central coastline and included sites within Island Beach State Park (IBSP), the Edwin B. Forsythe National Wildlife Refuge (EBFNWR), and the Great Bay Wildlife Management Area (GBWMA). IBSP is a preserved 3000 acre barrier island located on the Barnegat Peninsula that contains dense maritime forests, rolling sand dunes, and tidal marshes. Collection sites within IBSP included Spizzle Creek (SC) and Sedge Island (SI). SC is a tidal marsh creek located on the western side of the Southern Natural Area of IBSP. SI is a small 22-acre island located approximately 1.25 km west of the SC. EBFNWR contains more than 46,000 acres of protected coastal habitats; sampling occurred within the Barnegat Division of the refuge near or on Conklin Island, located in central Barnegat Bay. The area is comprised of salt

marshes and tidal creeks, with some sandy upland areas. GMWMA is a relatively pristine tidal salt marsh of the 5,500 acre peninsula located in at the southern end of Barnegat Bay. Sampling occurred along an 8.1 km paved road that runs through GMWMA and is utilized by nesting diamondback terrapins (Szerlag & McRobert 2006).

Field sampling and data analyses

Adult females were captured by hand on nesting areas or trapped using hoop or fyke nets during the reproductive seasons of 2006 (EBFNWR, SI), 2007 (EBFNWR, SC, and SI), and 2008 (EBFNWR, SI, and GBWMA). Carapace length, width, and height, plastron length and mass were recorded for all females sampled. Females were permanently marked by shell notching (Cagle 1939) and injected with a passive integrated transponder (PIT) tag. The reproductive status of female turtles was determined by palpation. X-radiographs were taken from many gravid females at EBFNWR, SC, and GBWMA (N = 67). Turtles were X-rayed at a 60 kV peak for 0.02 seconds from 0.5 m using a MinXray portable x-ray system. Some females were induced to oviposit as they were part of a paternity study (Chapter 4). These females were induced via interperotineal injection with 10-30 IU/kg Oxytocin (Ewert & Legler 1978). Other females naturally laid eggs in nests. Eggs mass, width, and length of all eggs laid were individually measured and recorded to the nearest 0.1 g and 0.1 mm. Because females did not always lay the entire clutch when induced with oxytocin, total clutch mass was determined by multiplying the total clutch size (as

determined by x-ray analysis (Gibbons & Greene 1979) by the mean mass of all eggs collected. Nests obtained by induction via oxytocin were incubated in a hatchery at the Lighthouse Center for Natural Resource Education in Waretown, NJ. All nests were protected with predator excluder cages. Mesh size of predator excluder cages prevented emerging hatchlings from escaping. Upon emergence, clutch success was recorded as the total number of hatchlings alive after the emergence of at least one hatchling divided by the total number of eggs incubated. Clutch success at Sedge Island was not calculated for this study because incubation conditions were experimentally manipulated (Wnek 2010). The remaining gravid females that were not induced were released at the point of capture after x-rays were taken. X-rays were analyzed to determine width of eggs and width of the pelvic aperture (Wilkinson & Gibbons 2005). Each egg width was measured to the nearest 0.1 mm using digital calipers. Pelvic aperture was measured as the shortest distance between the ilia (Wilkinson & Gibbons 2005) and recorded at the nearest 0.1 mm.

Statistical analyses of field data

We investigated within-clutch variation in egg mass to determine if our calculation of total clutch mass (mean egg mass multiplied by total number of eggs as determined by x-ray) was appropriate. We compared mean within-clutch variation in egg mass to variation in egg mass within all eggs sampled in all sites and years combined to determine if egg mass variation in the population was greater than egg mass variation within clutches. For all further analyzes we use mean clutch values of

egg mass, egg width, egg width, and egg sphericity. We combined all available data (all years and sites) within Barnegat Bay and determined if there were any significant linear correlations between the following variables: female carapace length, clutch size, egg mass, egg width, egg length, clutch mass, egg sphericity, pelvic width, and clutch success.

We tested for constraints on egg size several ways. First, we tested whether a quadratic function or a linear function better described the relationship between egg sphericity and female carapace length. Under the constraint hypothesis, we expected that a concave quadratic function to best describe the relationship because egg sphericity should increase with body size until the constraint is relaxed (Rollinson & Brooks 2008). Second, we tested whether linear and quadratic slopes better described the relationship between mean 1) egg width and female carapace length and 2) mean egg length and female carapace length. We standardized the data to use ANCOVA to compare the linear slopes of mean egg width and mean egg length as a function of female carapace length. Under the constraint hypothesis, we expected that the slope of egg width over body size should be greater than the slope of egg length over body size because egg width is likely the measure of egg size that is constrained (Congdon *et al.* 1983). Third, we tested whether pelvic width constrains egg width. We compared the slopes of mean clutch egg width and carapace length and pelvic width and carapace length using ANCOVA. We expected that if pelvic width constrains egg width, then the slopes would not differ (Wilkinson & Gibbons 2005). Fourth, we tested whether a reduction in egg sphericity would constrain clutch size. Because

female body size affects both clutch size and egg sphericity, we controlled for female carapace length using standardized residuals of the regression of clutch size and carapace length and of the regression of egg size and carapace length. We expected that if increased egg sphericity allows for increased packing efficiency, then there should be a positive relationship between egg sphericity and clutch size, after controlling for female carapace length.

We tested whether egg size was optimized in several ways. First, we compared the coefficient of variation for clutch size and egg mass. If the data are consistent with OES then we expected that the coefficient of variation would be greater for clutch size (Roosenburg & Dunham 1997). Second, we compared egg mass, clutch size, clutch mass, egg width, egg length, and female carapace length within sites between years and among sites within the same year using ANOVA and Tukey HSD tests. If the data are consistent with OES, we expected that any differences in reproductive output between years within sites would be due to changes in clutch size and not egg mass. If egg size is optimized in Barnegat Bay, then we expected that any differences in reproductive output within years between sites would be due to differences in clutch size rather than in measurements of egg size, except if there are constraints on egg size and the mean female size differs between sites. Third, we tested whether a quadratic or a linear function better described the relationship between egg mass and female carapace length. We expected under OES that egg mass would be described by a concave quadratic function because egg mass should increase with body size until the optimum egg mass is achieved (Rollinson &

Brooks 2008). Fourth, we tested whether a quadratic or a linear function better described the relationship between clutch size and female carapace length. Under OES, we predicted that clutch size would be best described as a linear function of female carapace length because when females optimize egg size, larger females should invest increased reproductive potential into producing more eggs not larger eggs. Fifth, we tested whether there was a significant tradeoff in clutch size and egg size. Because female body size affects both clutch size and egg size, we controlled for female carapace length using standardized residuals of the regression of clutch size and carapace length and of the regression of egg size and carapace length. Under OES, we expected a significant clutch size-egg size tradeoff.

Literature Survey

We surveyed the literature for available data on mean population female body size, clutch size, egg length, egg width, egg mass, and total clutch. We recorded standard deviations, standard errors, and ranges if available. We used female plastron length in our analysis of latitudinal variation in female body size, because it was the measure of female size most consistently reported. In some cases, means were estimated from graphs when actual values were not given. In one case, raw data on female plastron length were reported in tables (Roosenburg *et al.* 2009; Roosenburg *et al.* 2007; Roosenburg & Sullivan 2006) and we calculated the mean and standard deviation from these tables. Mean size of females reported in the literature are complicated by the fact that some are means of only nesting or gravid females (e.g.

Montevecchi & Burger 1975) while others are means of all females captured, including those of non-reproductive size (e.g Gibbons *et al.* 2001). When data was reported on both female sizes (including juveniles) and nesting female sizes, we recorded the latter. To test for the effects of latitude on mean population female plastron length, clutch size, egg mass, egg width, egg length, and total clutch mass we used regression analysis. Because clutch size, egg mass, egg width, egg length, and total clutch mass can also be affected by female body size, we also performed regression analyses for the effects of latitude using the standardized residuals of each variable on mean female population plastron length to provide body-size adjusted estimates of each variable. Finally, we tested for a tradeoff between mean population clutch size and egg size, after controlling for mean population female plastron length.

5.4 Results

Field study

The coefficient of variation of egg mass within clutches was smaller than the coefficient of variation for all eggs sampled within all sites (Table 5-3). Mean egg mass differed significantly between clutches within sites when eggs were weighed individually (EBFNWR: ANOVA, $F_{27,261}=26.72$, $P<0.001$; GBWMA: ANOVA, $F_{52,465}=83.244$, $P<0.001$; SC: ANOVA, $F_{31,343}=49.66$, $P<0.001$, SI: ANOVA, $F_{86,995}=36.73$, $P<0.001$). Since the data support the inference that egg mass varied little within-clutches, we proceeded to use mean egg mass within a clutch to calculate total clutch mass. In clutches where all eggs were weighed (N = 40, as indicated by x-ray),

there was a significant correlation between the sum total of all eggs measured individually and the calculated total clutch mass (mean egg mass multiplied by total eggs indicated on x-ray; $P<0.001$).

Within all clutches sampled, we found positive correlations between female carapace length and 1) clutch size, 2) egg mass, 3) egg width, 4) clutch mass, 5) egg sphericity, and 6) female pelvic width (Table 5-4). We found positive correlations between clutch size and 1) clutch mass, 2) egg sphericity, and 3) pelvic width (Table 5-4). We found positive correlations between egg mass and 1) egg width, 2) egg length, 3) clutch mass and 4) pelvic width (Table 5-4). We found positive correlations between egg width and 2) egg length, 3) clutch mass, 4) egg sphericity, and 5) female pelvic width (Table 5-4). We also found positive correlations between egg length and 1) clutch mass, 2) clutch mass, and 3) egg sphericity; we found positive correlations between clutch mass and pelvic width and between egg sphericity and pelvic width (Table 5-4). Negative relationships were found between egg length and 1) egg sphericity and 2) clutch size (Table 5-4). We found no relationship between clutch success and any clutch characteristic (Table 5-4).

A quadratic function did not better describe the relationship between female carapace length and mean clutch egg sphericity ($F_{2,190} = 2.05$, $P = 0.13$). A quadratic function did not better describe the relationship between female carapace length and mean clutch egg width (Figure 5-1a; $F_{2,190} = 0.80$, $P = 0.45$). Neither a linear ($P = 0.63$) nor a quadratic function ($P = 0.12$) significantly described the relationship between female carapace length and mean clutch egg length (Figure 5-1b). The linear

slope of female carapace length and mean clutch egg width was greater than the linear slope of female carapace length and mean clutch egg length (ANCOVA, $F_{1,383}$= 26.8, P< 0.001). The slope of female carapace length and pelvic width was greater than the slope of female carapace length and mean clutch egg width (ANCOVA, $F_{1,257}$ = 10.37, P =0.0014). There was no relationship between egg sphericity and clutch size when we controlled for female body size ($F_{1,155}$ = 1.08, P = 0.30).

Comparisons of the coefficient of variation for clutch size and egg mass indicated that clutch size varied about twice as much as egg mass for the majority of sites within years, except for EBFNWR in 2007 and SI in 2007 and 2008. Within EBFNWR, female carapace length, mean clutch egg mass, mean clutch egg length, and mean clutch egg width did not differ, but total clutch mass and clutch size were significantly smaller in 2008 (Table 5-5). On average clutch size was smaller by 2 eggs and clutch mass was 24 g less in 2008 (Table 5-5). Within SI, female carapace length, average clutch egg mass, total clutch mass, and clutch size did not differ, but eggs were shorter in length in 2008 and greater in width in 2006 (Table 5-6).

Within 2007, clutch size and female size did not differ between sampling locations (EBFNWR, SI, and SC), but females at EBFNWR had greater mean clutch egg mass, total clutch mass, mean clutch egg length, and mean clutch egg width (Table 5-7). Within 2008, mean clutch egg mass was not different between EBFNWR, SI, and GBWMA (Table 5-8). Females at GBWMA were smaller and had lower mean clutch egg width than females from EBFNWR. Females at GBWMA were smaller and had lower total clutch mass, smaller clutch sizes, but longer mean

clutch egg length when compared with females at SI (Table 5-8). Whereas, females at EBFNWR had smaller clutches with longer mean clutch egg length than females at SI (Table 5-8).

A quadratic function did not better describe the relationship between mean clutch egg mass and female carapace length ($F_{2,193} = 1.53$, $P = 0.22$; Figure 5-2a). A quadratic function did not better describe the relationship between clutch size and female carapace length ($F_{2,180} = 1.82$, $P = 0.16$; Figure 5-2b). We found a significant negative relationship between clutch size and egg size when we controlled for female body size ($F_{1,157} = 34.6$, $P < 0.001$; Figure 5-3).

Literature Survey

Data collected in the literature survey is summarized in Table 5-9. We found significant positive correlations between 1) population latitude and population mean clutch size and 2) population latitude and clutch mass (Table 5-10). We found significant negative correlations between population latitude and 1) population mean egg mass, 2) egg width, and 3) egg length (Table 5-10). There was no effect of latitude on population mean female plastron length (Table 5-10). When we performed analyses using body size adjusted estimates we found that there was no longer a relationship between population latitude and population mean clutch mass, but the relationships held between population latitude and population 1) clutch size, 2) mean egg mass, 3) egg width, and 4) egg length (Table 5-10). Furthermore, we found a

negative trade-off between population mean body size adjusted clutch size and population mean body size adjusted egg mass ($F_{1,4} = 26.1$, $P = 0.007$, $r = 0.93$).

5.5 Discussion

Our findings provide some support for body size specific constraints on egg shape and size in our nesting populations, but little support that maximum optimal egg size has been attained in larger females. First, we found that egg sphericity continued to increase with female body size. We did, however, find that the slope of egg width over carapace length increased at a faster rate than egg length over carapace length (Figures 5-1a and 5-1b), suggesting that egg width rather than egg length is constrained by female body size. However, the data did not support the inference that pelvic width constrains egg width because pelvic width over carapace length increased at a faster rate when compared to maximum egg width over carapace length. The data also did not support the inference that egg sphericity constrains clutch size. Egg width may be constrained by other morphological features, such as caudal gap (Clark et al 2001) or oviduct size and shape (Rose *et al.* 1996) and reduced egg sphericity may be constrained by the amount of calcium deposition required or enhanced water loss under desiccating conditions (Rose *et al.* 1996), which were not addressed in this study.

Our findings provide mixed support for OES in *M. terrapin* in Barnegat Bay, NJ. First, we expected and found that the coefficient of variation was generally twice as large for clutch size, but with some exceptions. We found that within sites between

years, differences in reproductive output (total clutch mass) were due to differences in clutch size but not egg mass, supporting OES (Tables 5-4 and 5-5). Differences in average clutch size and egg mass between sites within years did not follow a general trend and may be due to several factors such as differences in average female size and resource availability. Between sites within 2007, we found that differences in reproductive output (total clutch mass) were due to differences in egg mass, rather than differences in clutch size, which would not be expected under OES theory. In 2008, we found that differences in reproductive output between sites were due to differences in clutch size but not egg mass, supporting OES theory.

M. terrapin females in Barnegat Bay have not yet reached an optimum egg mass; mean egg mass increases with body size, but it does not do so at a decreasing rate (Figure 5-2a). *M. terrapin* females in Barnegat Bay have also not yet reached an optimum clutch size (Figure 5-2b) because clutch size does not increase with body size at a decreasing rate. Lastly, as predicted from OES, we found a negative relationship between clutch size and egg mass (Figure 5-3).

Our latitudinal analysis provides increased support for prior conclusions that females in the northern latitudes produce larger clutches with greater total clutch mass and that females in the southern latitudes produce eggs of larger mass, length, and width (Table 5-10). Although female body size did not vary with latitude, when we used body size adjusted clutch mass, clutch mass no longer varied with latitude (Table 5-10). The data indicate that the latitudinal variation in clutch mass was due to variation in female body size. Thus total reproductive output is determined by female

size not latitude and the characteristics of that output (clutch size and egg size) is determined by latitude and a negative tradeoff between clutch size and egg size.

Regardless of mean population female body size, females in the south on average produce smaller clutches with eggs of greater mass, width, and length. At least two scenarios in relationship to constraints on egg size are possible. In scenario 1, relationships between pelvic aperture width (or other features constraining egg size) and female body size are the same for northern and southern females and thus females in the north (an our population) do not have constraints on egg mass because females in the south are capable of producing larger eggs even though females are not larger. This is further supported by the fact that in Barnegat Bay, NJ the mean difference between female pelvic aperture width and maximum egg width is 4.6 mm (range 1.2-8.9, SD 1.6). In our population the mean egg width is 20.68 mm, while the largest recorded population mean egg width of 23.9 mm was measured in Comfort Island, Louisiana (Table 5-9), a difference of 3.2 mm. In Scenario (2) selection for larger pelvic aperture width (or other features constraining egg size) in the south might lead to a different relationship between the morphological feature constraining egg size and female body size than what was measured in Barnegat Bay. Thus egg size could be constrained by body size in both northern and southern females. The mean difference between maximum egg width and female pelvic aperture width in Barnegat Bay, NJ (4.6 mm) might reflect the space needed for soft tissue structures within the pelvic canal. Selection pressures favoring the development and passage of larger eggs in females of *Gopherus berlandieri*, *Kinosternon flavescens*, and

Terrapene ornate has lead proportionally larger pelvic canals in females than in males (Long & Rose 1989). Similarly, selection pressures could lead to proportionally larger pelvic canals of *M. terrapin* females from southern populations than in females from northern populations. Comparison of pelvic aperture width in females from southern and northern populations might resolve whether the relationship between pelvic aperture width and female size differs with latitudinal change. Experimental conditions in which females from southern and northern populations are raised in both southern and northern population conditions could elucidate whether northern females are capable of producing larger eggs and southern females of producing larger clutches. Alternatively, experimental manipulation of egg size in both northern and southern females might be possible through "allometric engineering" (Sinervo *et al.* 1992). In this process removal of some early stage follicles could decrease clutch size and should increase egg size because we would expect that the energy that would have normally been distributed equally among the entire clutch would then be distributed among the remaining follicles. Proper care should be taken when using "allometric engineering" because if there are indeed constraints on egg size, female with larger eggs could become eggbound or produce eggs at break during oviposition (Sinervo & Licht 1991b).

In conclusion, our data suggest that females in the north, including the Barnegat Bay population studied herein, are moving towards an optimal clutch size, while females south are likely optimizing egg size. Environmental variation between northern populations and southern populations might cause *M. terrapin* to vary

offspring size. Environmental variation is known to cause variation in parental investment (Congdon 1989; Congdon & Tinkle 1982; McGinley *et al.* 1987; Schultz 1991; Sinervo 1990). Females can alter parental investment by either producing many offspring by reducing offspring size or producing larger offspring by reducing clutch size. Larger, higher quality hatchling turtles might have increased survival rates (Congdon 1989). In *M. terrapin* hatchlings in southern populations have higher maintenance metabolism and utilize residual lipid stores at a faster rate than those in northern populations (Allman 2006). When reared in southern population conditions, hatchlings from small eggs have a lower survivorship than hatchlings from large eggs, but when reared in northern population conditions there is no difference in survivorship of hatchlings from small and large eggs (Allman 2006). Thus females in southern populations likely produce larger eggs to increase hatchling survivorship (Allman 2006). Females in northern populations may produce larger clutches rather than larger eggs to increase fecundity. The balanced mortality hypothesis (Fretwell 1969) predicts that larger clutch sizes in northern latitudes are a result of selection compensating for greater mortality rates in unpredictable environments (Price 1974; Tinkle 1961). Comparisons of hatching success and hatchling mortality rates along a longitudinal gradient might provide additional insight as to why northern females produce larger clutches in northern latitudes.

Table 5-1. Literature Review of relationships between female body size, clutch size, clutch mass, egg size, and pelvic aperture width in *Malaclemys terrapin*. Sources are as follows (1) Montevecchi and Burger 1975, (2) Seigel 1980, (3) Zimmerman 1989, (4) Goodwin 1994, (5) Roosenburg and Dunham 1997, and (6) Allman 2006.

Relationship	+ Correlation	No Correlation
Female PL X Clutch Size	1,2,4,5	3
Female PL X Clutch Mass	1	
Female PL X Egg Mass		1,2,5
Female PL X Egg Length		1,2
Female PL X Egg Width		1,2,3
Female PL X Nest Depth		1,4
Female PL X Nest Success		4
Female PL X Pelvic Aperture Width	3	
Clutch Size X Clutch Mass	1,3	
Clutch Size X Egg Mass		1,2,5
Clutch Size X Egg Length		1,2
Clutch Size X Egg Width		1,2
Clutch Size X Egg Elongation		1
Clutch Mass X Egg Mass		1
Egg Mass X Egg Length	3,6	
Egg Mass X Egg Width	3,6	
Egg Length X Egg Width	1,3	
Pelvic Aperture Width X Egg Width		3

Table 5-2. Latitudinal trends in female size, clutch size, and egg size characteristics in *Malaclemys terrapin* .

Measurement	Latitudinal Trend	Statistical Test	# Populations	Author
Female plastron length	not significant	ANOVA	3	Zimmerman 1989
	suggested larger in north	none	3	Goodwin 1994
Total clutch mass	not significant	ANOVA	3	Zimmerman 1989
	significantly less in the south	not given	3	Allman 2006
Clutch Size	larger in north	no test given	5	Seigel 1980
	significantly larger in north	ANOVA, Tukey Post Hoc	3	Zimmerman 1989
	suggested larger in north	none	3	Goodwin 1994
	significantly larger in north	ANOVA	3	Allman 2006
Egg length	significantly larger in south	t-test	4	Seigel 1980
	significant increase from north to south	ANOVA, Tukey Post Hoc	3	Zimmerman 1989
	suggested increase from north to south	none	3	Goodwin 1994
	significantly larger in south	ANOVA	3	Allman 2006
Egg width	suggested increase from north to south	none	3	Goodwin 1994
	significantly smaller in north	ANOVA, Tukey Post Hoc	3	Zimmerman 1989
	egg width did not vary	ANOVA	3	Allman 2006
Egg Mass	significantly larger in south	t-test	2	Seigel 1980
	signficant increase from north to south	ANOVA, Tukey Post Hoc	3	Zimmerman 1989
	significantly larger in south	ANOVA	3	Allman 2006

Table 5-3. Summary statistics of egg-mass variation within clutches and among all diamondback terrapin eggs categorized by site in Barnegat Bay, NJ.

	Source	n	Mean egg mass	SD	CV
Within clutches	All Sites	200	7.99	0.48	0.06
	EBFNWR	28	8.73	0.65	0.07
	GBWMA	53	7.89	0.33	0.04
	SC	32	7.72	0.42	0.05
	SI	87	7.91	0.54	0.07
Among all eggs	All sites	2264	7.97	1.21	0.15
	EBFNWR	289	8.72	1.30	0.15
	GBWMA	518	7.89	1.08	0.14
	SC	375	7.69	0.98	0.13
	SI	1082	7.90	1.24	0.16

Table 5-4. Linear correlations of female and clutch characteristics for all nests studied in Barnegat Bay, NJ (2006-2008). All significant correla
bold. Negative r values indicate a negative relationship.

	n	X	STD	range	Carapace Length	Clutch Size	Egg Mass	Egg Width	Egg Length	Clutch Mass	Egg Sphericity
Carapace Length	224	184.39	14.33	145-220	--	**r = 0.463 p < 0.001**	**r = 0.431 p < 0.001**	**r = 0.526 p < 0.001**	r = 0.035 p = 0.63	**r = 0.689 p < 0.001**	**r = 0.390 p < 0.001**
Clutch Size	191	12.06	2.76	5-19	--	--	r = -0.101 p = 0.195	r = 0.009 p = 0.911	**r = -0.287 p < 0.001**	**r = 0.797 p < 0.001**	**r = 0.202 p = 0.009**
Mean Egg Mass (g)	201	7.99	1.09	5.9-11.3	--	--	--	**r = 0.741 p < 0.001**	**r = 0.562 p < 0.001**	**r = 0.506 p < 0.001**	r = 0.06 p = 0.397
Mean Clutch Egg	201	20.85	1.22	18.5-24.8	--	--	--	--	**r = 0.398 p < 0.001**	**r = 0.460 p < 0.001**	**r = 0.421 p < 0.001**
Mean Clutch Egg	201	31.22	2.15	24.6-36.5	--	--	--	--	--	**r = 0.187 p = 0.016**	**r = -0.661 p < 0.001**
Clutch Mass (g)	166	98.23	23.55	28.5-147.4	--	--	--	--	--	--	**r = 0.18 p = 0.02**
Mean Clutch Egg	201	0.67	0.05	0.56-0.80	--	--	--	--	--	--	--
Pelvic Width	67	27.62	2.22	23.5-33	--	--	--	--	--	--	--
Clutch Success	116	70	31.5	0-100	--	--	--	--	--	--	--

Table 5-5. Variation in female carapace length, egg mass, clutch mass, clutch size, egg length, and egg width from *M. terrapin* nests among years at the Edwin B. Forsythe National Wildlife Refuge (EBFNWR). Clutch size was determined by x-radiographs. Clutch mass was calculated as average egg mass multiplied by total clutch size. Average egg mass, width, and length were utilized from each clutch. Values given are mean, standard error (SE), coefficient of variation (CV), and total number of females or nests sampled (N). Results of ANOVA are given. Results from 2006 nests were not used in the analysis because of low sample size (N = 2).

measurement	**year**	**mean**	**SE**	**CV**	**N**	**ANOVA**
Female carapace length (mm)	2007	193.94	2.91	0.06	17	P = 0.45
	2008	190.27	3.92	0.07	11	
Average Clutch Egg Mass (g)	2007	8.95	0.27	0.12	17	P = 0.21
	2008	8.40	0.33	0.13	11	
Total Clutch Mass (g)	2007	116.51	4.55	0.14	13	P = 0.019
	2008	92.88	8.80	0.30	10	
Clutch Size	2007	13.08	0.51	0.14	13	P = 0.056
	2008	11.00	0.97	0.28	10	
Average Clutch Egg Length (mm)	2007	32.64	0.35	0.04	17	P = 0.46
	2008	33.06	0.43	0.04	11	
Average Clutch Egg width (mm)	2007	21.77	0.24	0.05	17	P = 0.12
	2008	21.16	0.29	0.05	11	

Table 5-6. Variation in female carapace length, clutch size, egg mass, clutch mass, egg width, and egg length from nests among years at Sedge Island (SI). Clutch size and clutch mass were the total count and total mass of all eggs in the naturally deposited nest chamber. Egg mass, width, and length were individually measured for all eggs. Values given are the mean, standard error (SE), coefficient of variation (CV), and total number of females or nests sampled (N). Results of ANOVA and post hoc analysis are given.

	year	mean	SE	CV	N	ANOVA	Tukey Test	
Female	2006	190.20	3.34	0.07	15		2006 = 2007	P>0.05
carapace	2007	187.75	1.70	0.05	36	P = 0.84	2006 = 2008	P>0.05
length (mm)	2008	188.24	3.10	0.09	29		2007 = 2008	P>0.05
Average	2006	7.83	0.34	0.17	16		2006 = 2007	P>0.05
Clutch Egg	2007	7.82	0.17	0.13	38	P = 0.64	2006 = 2008	P>0.05
Mass (g)	2008	8.06	0.18	0.13	32		2007 = 2008	P>0.05
	2006	97.30	5.98	0.25	16		2006 = 2007	P>0.05
Total Clutch	2007	98.76	3.65	0.22	37	P = 0.12	2006 = 2008	P>0.05
Mass (g)	2008	108.24	3.41	0.18	32		2007 = 2008	P>0.05
	2006	12.45	0.59	0.21	20		2006 = 2007	P>0.05
Clutch Size	2007	12.65	0.35	0.17	40	P = 0.19	2006 = 2008	P>0.05
	2008	13.48	0.38	0.16	33		2007 = 2008	P>0.05
Average	2006	31.94	0.37	0.05	16		2006 = 2007	P>0.05
Clutch Egg	2007	31.55	0.23	0.04	38	P < 0.001	2006 ≠ 2008	P<0.01
Length (mm)	2008	29.38	0.23	0.04	32		2007 ≠ 2008	P<0.01
Average	2006	22.86	0.28	0.05	16		2006 ≠ 2007	P<0.01
Clutch Egg	2007	20.75	0.18	0.05	38	P < 0.001	2006 ≠ 2008	P<0.01
width (mm)	2008	20.57	0.18	0.05	32		2007 = 2008	P>0.05

Table 5-7. Variation in female carapace length, egg mass, clutch mass, clutch size, egg length, and egg width from *M. terrapin* nests between sites (EBFNWR, SI, and SC) in 2007. Clutch size was determined by x-radiographs from clutches at EBFNWR and SC (Spizzle Creek) and from naturally deposited nests at SI. Clutch mass at EBFNWR and SC was determined by average egg mass multiplied by total clutch size and at SI by total mass of all eggs deposited. Average egg mass, width, and length were utilized from each clutch. Values given are the mean, standard error (SE), coefficient of variation (CV), and total number of females or nests sampled (N). Results of ANOVA and Tukey HSD analysis are given.

measurement	location	mean	SE	CV	N	ANOVA	Tukey HSD Test	
Female	EBFNWR	193.94	2.91	0.06	17		SI = EBFNWR	P>0.05
carapace length	SI	187.75	1.70	0.05	36	P = 0.14	SI = SC	P>0.05
(mm)	SC	187.56	2.24	0.07	32		EBFNWR = SC	P>0.05
	EBFNWR	8.95	0.27	0.12	17		SI≠EBFNWR	P<0.01
Mean Clutch	SI	7.82	0.17	0.13	38	P = 0.0002	SI=SC	P>0.05
Egg Mass (g)	SC	7.72	0.16	0.12	32		EBFNWR≠SC	P<0.01
	EBFNWR	116.51	4.55	0.14	13		SI≠EBFNWR	P<0.05
Total Clutch	SI	98.76	3.65	0.22	37	P = 0.037	SI=SC	P>0.05
Mass (g)	SC	103.87	4.82	0.20	19		EBFNWR=SC	P>0.05
	EBFNWR	13.08	0.51	0.14	13		SI=EBFNWR	P>0.05
Clutch Size	SI	12.65	0.35	0.18	39	P = 0.84	SI=SC	P>0.05
	SC	12.56	0.70	0.28	25		EBFNWR=SC	P>0.05
Mean Clutch	EBFNWR	32.64	0.35	0.04	17		SI≠EBFNWR	P<0.05
Egg Length	SI	31.55	0.23	0.04	38	P = 0.030	SI=SC	P>0.05
(mm)	SC	31.74	0.25	0.04	32		EBFNWR=SC	P>0.05
Mean Clutch	EBFNWR	21.77	0.24	0.05	17		SI≠EBFNWR	P<0.01
Egg width	SI	20.75	0.18	0.05	38	P<0.0001	SI=SC	P>0.05
(mm)	SC	20.38	0.16	0.04	32		EBFNWR≠SC	P<0.01

Table 5-8. Variation in female carapace length, egg mass, clutch mass, clutch size, egg length, and egg width from *M. terrapin* nests between sites (EBFNWR, SI, and GBWMA) in 2008. Clutch size was determined by x-radiographs. Clutch mass was determined by average egg mass multiplied by total clutch size. Average egg mass, width, and length were utilized from each clutch. Values given are the mean, standard error (SE), coefficient of variation (CV), and total number of females or nests sampled (N). Results of ANOVA and post-hoc analysis are given.

measurement	location	mean	SE	CV	N	ANOVA	Tukey HSD Test	
Female	EBFNWR	190.27	3.92	0.07	11		EBFNWR = SI	< 0.01
carapace length	SI	188.24	3.10	0.09	29	P < 0.0001	EBFNWR ≠ GBWMA	> 0.05
(mm)	GBWMA	173.82	1.35	0.06	68		SI ≠ GBWMA	< 0.01
	EBFNWR	8.40	0.33	0.13	11		EBFNWR = SI	> 0.05
Mean Clutch	SI	8.06	0.18	0.13	32	P = 0.31	EBFNWR = GBWMA	> 0.05
Egg Mass (g)	GBWMA	7.89	0.14	0.13	53		SI = GBWMA	> 0.05
	EBFNWR	92.88	8.80	0.30	10		EBFNWR = SI	> 0.05
Total Clutch	SI	108.24	3.41	0.18	32	P < 0.0001	EBFNWR = GBWMA	> 0.05
Mass (g)	GBWMA	85.07	3.21	0.23	37		SI ≠ GBWMA	< 0.01
	EBFNWR	11.00	0.97	0.28	10		EBFNWR ≠ SI	< 0.05
Clutch Size	SI	13.48	0.38	0.16	33	P < 0.0001	EBFNWR = GBWMA	> 0.05
	GBWMA	10.56	0.33	0.22	50		SI ≠ GBWMA	< 0.01
Mean Clutch	EBFNWR	33.06	0.43	0.04	11		EBFNWR ≠ SI	< 0.01
Egg Length	SI	29.38	0.23	0.04	32	P < 0.0001	EBFNWR = GBWMA	> 0.05
(mm)	GBWMA	32.60	0.21	0.05	53		SI ≠ GBWMA	< 0.01
Mean Clutch	EBFNWR	21.16	0.29	0.05	11		EBFNWR = SI	> 0.05
Egg width	SI	20.57	0.18	0.05	32	P = 0.046	EBFNWR ≠ GBWMA	< 0.05
(mm)	GBWMA	20.36	0.13	0.05	53		SI = GBWMA	> 0.05

Table 5-9. Latitudinal data on mean population female size and nesting characteristics of *Malaclemys terrapin* . Ranges and standard deviation (STD) or standard error (SE) in parentheses. The ≈ indicates that data were estimated from graphs or raw data tables.

LAT	Location	plastron length (mm)	clutch size	egg length (mm)	egg width (mm)	egg mass (g)	total clutch mass (g)	References
25.27	Big Sable Creek, FL	160 (SD 12)						Hart & McIvor 2008
25.85	S. Florida Bay, FL	181	5.82					Baldwin et. al 2005
28.35	Merritt Island, FL	154 (SD 10.0)	6.7 (SD 1.4)	39.0 (SD 1.3)	22.3 (SD 1.2)	12.48 (SD 0.73)	75.3 (SD 13.1)	Seigel 1979, Seigel 1980
29.49	Galveston Bay, TX	≈160 (SD 10)	7 (n=1)	39 (n=1)	23 (n=1)			Hogan 2003
29.84	Comfort Island, LA		8.5 (SD 2.2)	37.3 (SD 1.67)	23.9 (SD 1.05)			Burns & Williams 1972
30.56	Nassau County, FL	177.3	6.7 (SD 1.4)	37.2 (SD 1.7)	22.7 (SD 1.1)	11.5 (SD 1.4)		Butler 2000, 2002, Butler et al. 2004
32.62	Kiawah Island, SC	157 (SD 12.1)	6.9 (SD 1.5)	36.9 (SD 2.3)	22.2 (SD 1.3)	11.2 (SD 0.8)	76.5 (SD 19.5)	Zimmerman 1989
32.74	Grice Cove, SC		6.0 (SE 0.38)	36.3 (SE 0.12)	21.8 (SE 0.08)	10.4 (SE 0.08)	62.4 (SE 4.2)	Allman 2006
32.74	Grice Cove, SC	152 (SE 0.15)						Estep 2005
32.79	Charleston, SC	158 (SE 0.091)						Lee 2003
37.23	Yorktown, VA	175 (n=1)	7 (n=1)					Reid 1955
38.44	Paxtuxent River, MD		12.29 (SE 0.13)			9.87 (SE 0.08)	128.21 (SE 1.75)	Roosenburg & Dunham 1997
38.44	Paxtuxent River, MD			32.4 (SD 2.64)	21.6 (SD 1.46)	9.1 (SD 1.81)		Roosenburg & Kelly 1996
38.44	Paxtuxent River, MD			34.8 (SD 1.68)	22.3 (SD 1.18)	10.29 (SD 1.41)		Roosenburg & Dennis 2005
38.44	Paxtuxent River, MD		12.2 (SE 0.42)	34.3 (SE 0.13)	21.2 (SE 0.09)	9.9 (SE 0.09)	121.2 (SE 4.4)	Allman 2006
38.44	MD	175	12 (n=1)	31.1 (28.5-35.0)	21.2 (20.0-22.5)			McCauley 1945
38.77	Poplar Island, MD	≈195 (SD 9.4)	13.11 (SE 0.241)			9.86 (SE 0.086)	127.4 (SE 2.502)	Roosenburg et al. 2006, 2007, 2009
39.04	Stone Harbor, NJ		(5-23)	32.2 (SD 2.0)		7.3 (SD 1.25)		Herlands et al. 2004
39.49	Little Beach, NJ	154.4 (SD 9.9)	9.76 (SD 2.61)	31.6 (26.0-36.5)	19.7 (SD 1.1)	7.7 (SD 1.1)	71.8 (SD 17.7)	Burger 1977, Montevecchi & Burger 1975
39.52	Great Bay, NJ	158						Sverlag & McRobert 2006
39.75	Barnegat Bay, NJ	165.7 (SD 11.7)	12.06 (SD 2.76)	31.2 (SD 2.15)	20.85 (SD 1.22)	8.0 (SD 1.09)	98.23(SD 23.55)	This study
40.45	Sandy Hook, NJ	178.1 (SD 11.0)	13.27 (SD 3.7)					Ner 2003
40.61	Jamaica Bay, NY	172.9 (SD 8.63)	10.9 (SD 3.53)					Feinburg & Burke 2003, Feinburg 2000
40.61	Jamaica Bay, NY		11.8 (SD 3.1)					Giambanco 2002
40.61	Jamaica Bay, NY	171.9 (SD 11.1)	12.86 (SD 2.66)					Ner 2003
40.87	Oyster Bay, NY	≈180 (SD 10)						Baur 2004
41.27	Madison, CT	164.6 (SD 7.14)	9.6 (SD 3.58)					Aresco 1996
41.74	Barrington, RI	200 (175-225)	15.8 (2-21)	32.1 (SD 1.9)	21.1 (SD 1.1)			Goodwin 1994
41.74	Barrington, RI		16.1 (SE 0.65)	32.9 (SE 0.13)	20.9 (SE 0.08)	8.4 (SE 0.08)	135.6 (SE 7.1)	Allman 2006
41.94	Wellfleet, MA	164	12 (4-22)	27.1		7.75 (4.5-11)		Lewis in Brennessal 2006

Table 5-10. Effect of latitude on population mean female plastron length, clutch size, egg mass, egg width, egg length, and clutch mass in *M. terrapin* (row 1). Effect of latitude on body size adjusted population clutch size, egg mass, egg width, egg length, and clutch mass (row 2). Values in bold indicate a significant correlation ($P < 0.05$). Negative r values indicate a negative correlation.

	Plastron Length	Clutch Size	Egg Mass	Egg Width	Egg Length	Clutch Mass
r	0.32	0.82	-0.90	-0.80	-0.87	0.67
p-value	0.16	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.048**
r	--	0.89	-0.96	-0.83	-0.87	0.08
p-value	--	**<0.0001**	**0.001**	**0.01**	**0.002**	0.89

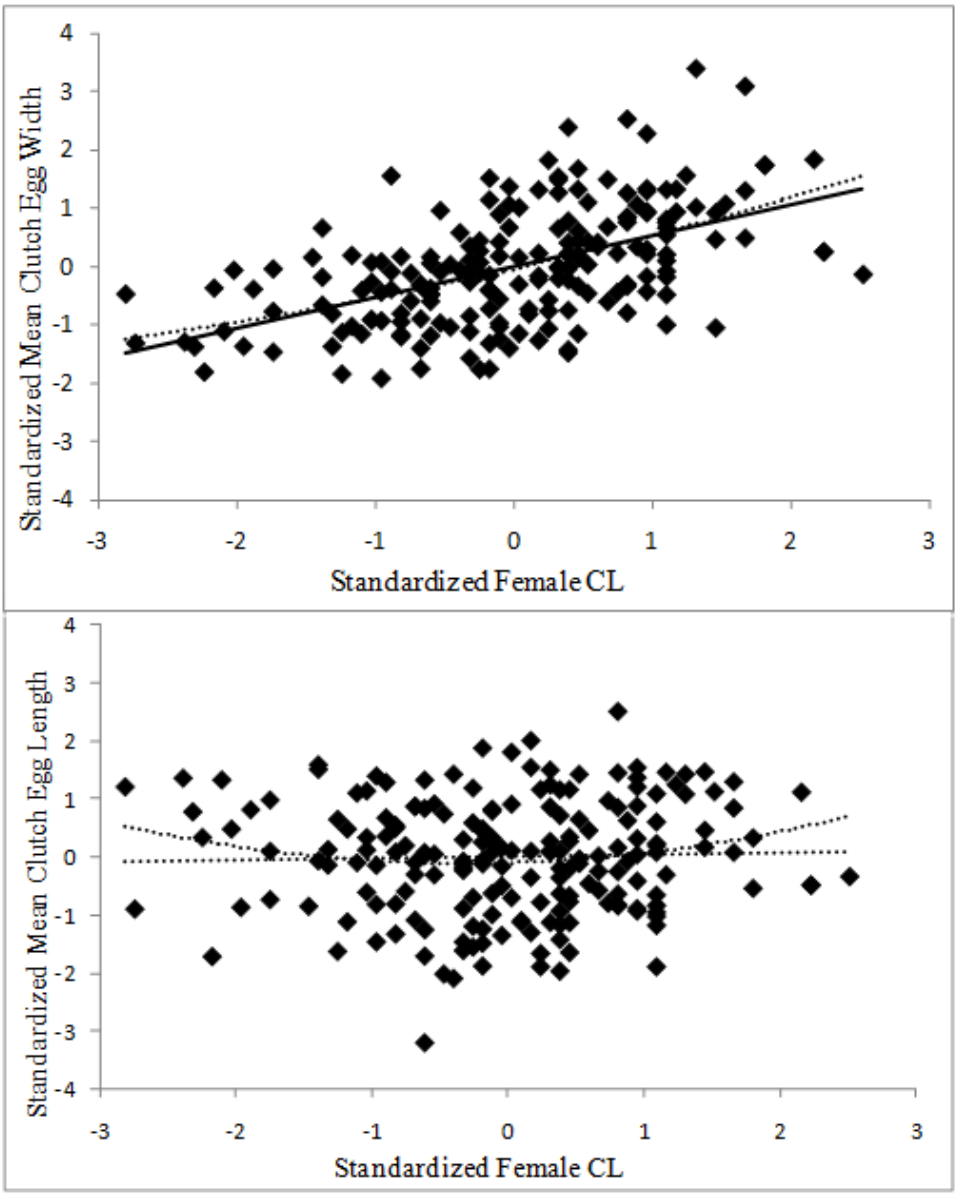


Figure 5-1a-b. Quadratic and linear relationships between standardized female carapace length and standardized mean clutch egg width (1a) and standardized mean clutch egg length (1b). In 1a, the quadratic equation did not have more support than the linear equation ($F_{2,190} = 0.80$, $P = 0.45$). In 1b, neither the linear nor the quadratic equations had significant support.

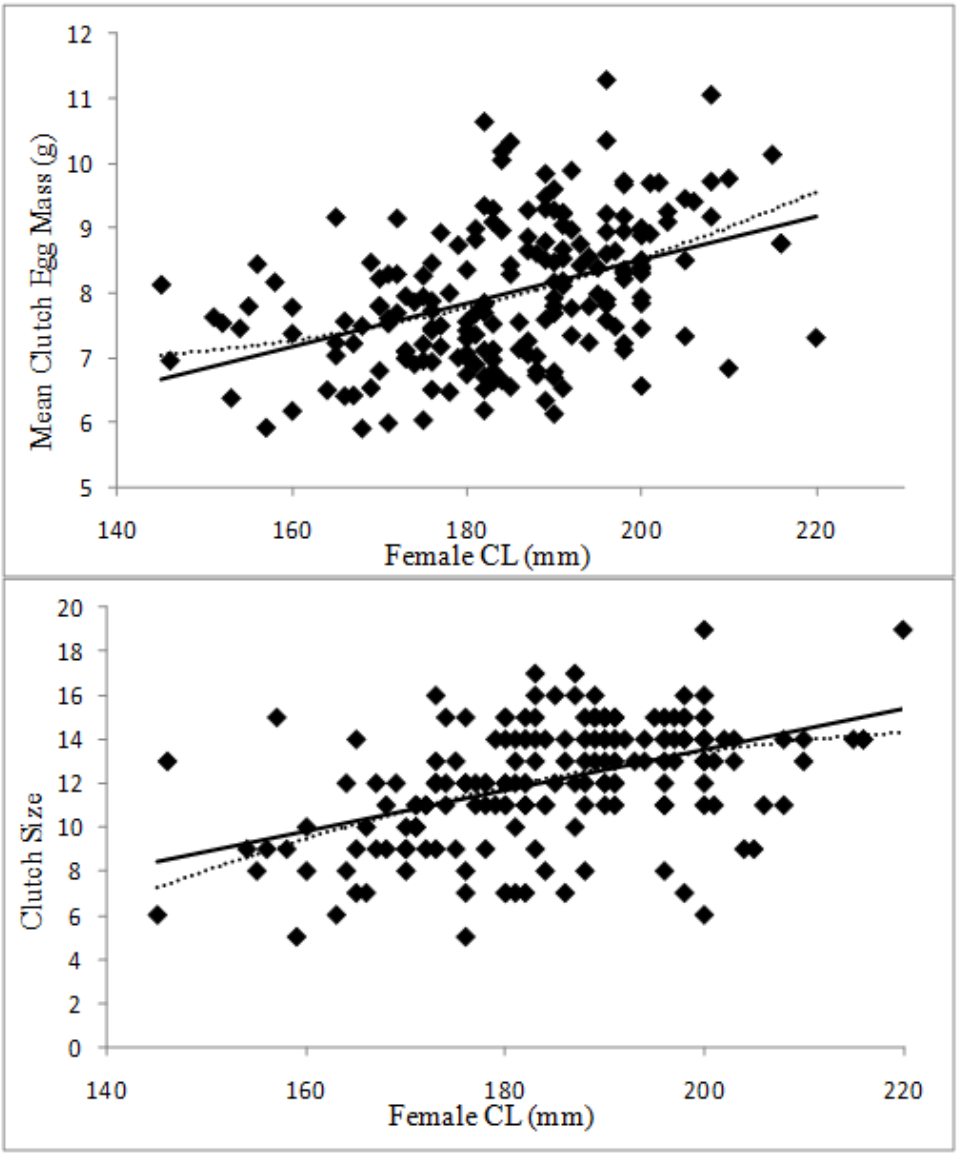


Figure 5-2a-b. Relationship between a) carapace length and mean clutch egg mass and b) carapace length and clutch size. a) A quadratic regression described the relationship between female carapace length and mean clutch egg mass better than a linear regression ($F_{2,193} = 1.53$, $P = 0.22$). b) Both the linear and quadratic functions fit significantly, however the quadratic function was not a significantly better fit ($F_{2, 180} = 1.82$, $P = 0.16$).

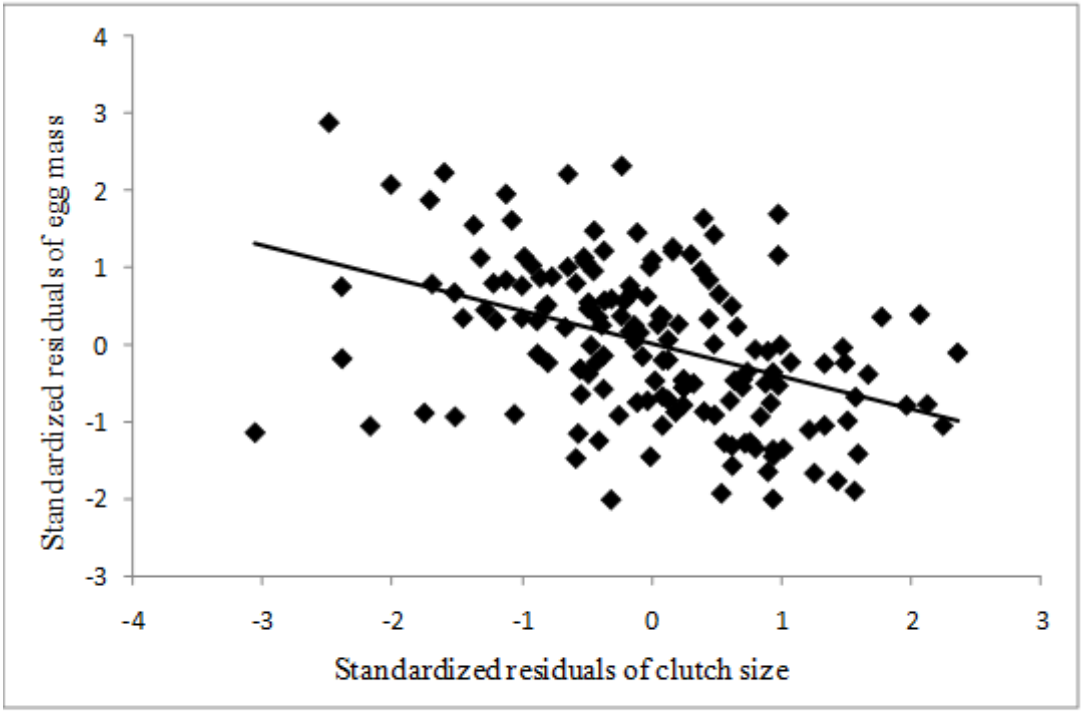


Figure 5-3 Relationship between clutch size and average egg mass when controlling for female carapace length. A significant negative relationship suggests a trade-off between clutch size and egg size ($F_{1,157} = 34.6$, $P<0.001$).

CHAPTER 6: Dissertation summary and applications

6.1 Introduction

This dissertation examined the dispersal, mating system, and reproductive output of the diamondback terrapin (*Malaclemys terrapin*) and how they affect population genetic diversity and how they are affected by anthropogenic activities. Combining spatial, mark-recapture, and genetic analyses, this dissertation has provided the most complete picture of the dispersal and mating system of the terrapin to date. I have provided information on the demographic (i.e. sex ratio) and ecological (landscape features) factors affecting the diamondback terrapin in Barnegat Bay as well as characterized the spatial structure and mating system. I addressed fundamental questions that are relevant to terrapin conservation and have laid the groundwork for future research on landscape genetics, sex-biased dispersal, natal philopatry, mating systems, sexual selection, and sperm storage in the terrapin. This dissertation sheds new light on the dispersal and mating behavior of these reptiles endemic to estuarine habitats and advances the current knowledge of mating behaviors in relation to population sex ratios.

6.2 Summary of major findings

The major findings from the previous four chapters are briefly summarized as follows:

1) **CHAPTER 2: Landscape genetic structure of the diamondback terrapin (*Malaclemys* terrapin) in a highly fragmented ecosystem**

Diamondback terrapins on the central coast of New Jersey exhibit low, yet significant levels of fine-scale spatial genetic structure. Isolation by distance was not evident. Fine-scale genetic structuring appears to be affected by habitat fragmentation, despite the aquatic connectivity between sampling locales and the semi-aquatic lifestyle of the terrapin. Estuarine emergent wetland was identified as the primary landscape feature affecting gene flow. Other landscape features may currently affect dispersal, but lag times in genetic signatures due to large population sizes and long generation times could mask their influence on terrapin dispersal.

2) **CHAPTER 3: Sex-biased dispersal, natal philopatry, and home range movements of the diamondback terrapin, *Malaclemys terrapin***

Capture-mark-recapture analysis indicated that all individuals have relatively small dispersal distances (<2 km), with mature females dispersing greater distances than males, both immature and mature, but not further than immature females. Mean assignment indices, spatial autocorrelation, and first-generation migrant tests indicated that mature males exhibited sex-biased dispersal and mature females exhibited natal philopatry to nesting beaches. When compared to males, a larger number of females were identified as first-generation migrants. However, males had a greater tendency to disperse in our female-biased populations.

Although capture-mark-recapture studies (including ours) indicate high fidelity for both males and females, we reconcile our results by noting the temporal and spatial restrictions of capture-mark-recapture studies. In particular, smaller individuals are often not captured in these studies and thus it is possible that some males disperse away from their natal area (or into the study area) before capture-mark-recapture studies have marked them. In otherwords, the individuals might only be captured after they have already dispersed away from their natal area and after they have established small home ranges.

3) **CHAPTER 4: Inter-population variation of multiple paternity in the diamondback terrapin, *Malaclemys terrapin***

Frequency of multiple paternity differed significantly among locations, ranging from 12.5 to 45.7%. Clutches with multiple paternity did not differ from clutches with single paternity with respect to female size, clutch size, egg size, hatchling size, or hatching success, suggesting that multiple paternity may not provide initial benefits to offspring survivorship. Male and females mated within their home ranges and thus mating events are not responsible for the high levels of gene flow documented in this species. Terrapins stored sperm storage both within and between seasons. Frequency of multiple paternity exhibited a

significant non-linear correlation with population sex ratio and may be related to sexual selection, availability of mates, and sperm storage.

4) **CHAPTER 5: Constraints on egg size, optimal egg size theory, and latitudinal reproductive variation in the diamondback terrapin (*Malaclemys terrapin*)**

Female body size and egg size were correlated suggesting a constraint on egg size in *M. terrapin*. However, pelvic aperture width did not increase at the same rate as egg width on female body size, suggesting that pelvic aperture width is not the main cause of egg width constraint. There was a trade-off between clutch size and egg size in *M. terrapin* supporting optimal egg size theory, but the data also supports optimization of clutch size not egg size in *M. terrapin* of Barnegat Bay, NJ. Latitudinal variation in clutch size and egg size indicate that clutch size is optimized in northern latitudes, and egg size is optimized in southern latitudes. Clutch size variation within sites increases with latitude. Variation in reproductive output on nesting beaches in northern latitudes could significantly increase gene correlations on a nestings beaches in northern latitudes in comparison to nesting beaches in southern latitudes, especially when there is also high variation in hatching success (due to predation, flooding, etc.) and natal philopatry.

6.3 Conservation Implications

The behavioral and genetic results of this dissertation can be applied toward conservation management of the terrapin in many ways. My landscape genetic analysis (Chapter 2) identified estuarine emergent wetland (Figure 2-2) as a feature necessary for gene flow and the least-cost path which best explained dispersal paths for terrapins can be used to inform managers in developing conservation plans. The utilization of least-cost models from genetic data can significantly improve the quality of and confidence in models of dispersal, migration and connectivity and such models have been employed world-wide to plan landscape-scale conservation strategies and design reserves (Epps *et al.* 2007). The least-cost paths developed in this dissertation can be used to identify corridors that are necessary for dispersal and can inform managers as to the best locations to maintain habitat, enhance habitat, or prevent further habitat destruction, especially along the estuarine shoreline.

A corridor is a strip of land intended to allow passage by a particular wildlife species between two or more wildland areas (Beier *et al.* 2008). Corridor design generally follows three basic steps. First, researchers indentify the landscape and focal species to define their biological goals (Beier *et al.* 2008). Second, the resistance or costs of different landscape types are determined for that species (Beier *et al.* 2008). Finally, the researcher determines which strips of land provide the lowest cost between wildlands to determine the corridor design for the focal species (Beier *et al.* 2008). Beir *et al.* (2008) promote the use of linkage design (connective land intended to promote movement of multiple focal species) over corridor developed for

a single species. Linkage designs that combine the corridor designs of multiple and diverse focal species are more likely to encompass the true least-cost corridor of each focal species despite uncertainty and they are also more likely to serve other species sharing traits with the suite of focal species (Beir *et al.* 2009). Thus, it should be a high priority to develop corridor designs for other focal species inhabiting the Barnegat Bay Estuary in order to develop linkage designs between the local, state, and federally protected habitats (e.g. Edwin B. Forsythe Wildlife Refuge and the Great Bay Wildlife Management Area).

National, state, and local organizations and programs such as the Nature Conservancy, the Trust for Public Land, NJ Natural Lands Trust, NJDEP Green Acres Program, Garden State Preservation Trust, The Land Conservancy of NJ, Ocean County Natural Lands Trust Program, and the Barnegat Bay National Estuary Program could make use of my landscape model to help determine priority parcels of land to protect in order to prevent further destruction of estuarine emergent wetland.

Although not an exhaustive list, the following provide examples of barriers to terrapin movement identified by the best-fit least-cost landscape model (Chapter 2, Fig 2-2):

1. **Beach Haven West:** a privately owned residential community with extensive man-made channelization but lacking estuarine emergent wetland along the shoreline.

2. **Seaside Heights, Seaside Park, and Lavallette:** privately owned residential communities with extensive development but lacking estuarine emergent wetland along the shoreline (Figure 6-2).
3. **Sunrise Beach:** a privately owned residential community with extensive man-made channelization but lacking estuarine emergent wetland along the shoreline.
4. **Lanoka Harbor:** a privately owned residential community with extensive man-made channelization but lacking estuarine emergent wetland along the shoreline.
5. **Island Beach State Park:** protected state lands located on a barrier island, but lacking emergent wetland in some areas along the shoreline (Figure 6-2).
6. **Big Creek, Tuckerton:** intersection of protected county (Ocean County Natural Lands Trust Program), state (Great Bay Wildlife Management Area), and federal lands (Edwin B. Forsythe National Wildlife Refuge). The intersection of these protected lands is a small area with estuarine emergent wetland, but special emphasis is needed to maintain the habitat and possibly to expand the size of the area. The Ocean County Natural Lands Trust Program has just recently approved the acquisition of Osborn Island, 96 acres of maritime forest directly southwest of the Big Creek. Simple real estate searches on the web indicate additional acres of land are

for sale in this area and many are already NJDEP approved for development.

7. **Dock Street to Seameadow Lane, Parkertown**: property in public ownership sandwiched between lands protected in the Edwin B. Forsythe Wildlife Refuge, many of which are currently for sale and possess NJDEP approval for development.

Protecting both males and females during dispersal events is key to maintaining gene flow (Chapter 3). Because the mixed model analysis (Chapter 2) identified open water and development as the primary landscape features impeding gene flow (Figure 2-3), it would be beneficial to manage protected land and adjacent waterways by methods that would prevent injury or mortality to males and females when they are utilizing these habitats. Several known threats include interactions with commercial style crab pots, boats, and motor vehicles.

Male and females terrapins often encounter commercial style crab pots in shallow and deeper water habitats. Crab pots often become lost or abandoned ("ghost pots"). These pots can continue to catch terrapins long after they have been lost. At or above 20^{o} C terrapins drown in 2 to 4 hours (Roosenburg *et al.* 1997). Pots are typically only checked once within a 24 hour period, resulting in the drowning death of many terrapins (Roosenburg *et al.* 1997). In New Jersey, pots in waterways less than 150 feet wide, require terrapin excluders devices (TEDs) of 5 by 15 cm (Watters 2004). Although, TEDs of this size are extremely useful in preventing large female

terrapins from entering the pots, males and small females are still able to enter (personal observation, see Figure 6-3). Within the Barnegat Bay system, there have not been any studies implemented to determine the number of abandoned crab pots or the total impact of these abandoned pots on the terrapin population. However, during our 2006-2009 capture-mark-recapture efforts in Barnegat Bay over 50 abandoned crab pots were removed from the North Forsythe study area. Approximately 30 terrapins (males and females) were removed from the abandoned pots and over half of them had drowned (Avery, unpublished). Furthermore, in 2007 one mature female that was equipped with radio-telemetry equipment was recovered washed up along the shoreline of Gunning River between the intersection of North Forsythe and South Forsythe. The female's physiological condition indicated that her cause of death was drowning. Gunning River is popular deep water (4-5 meters) location for recreational and commercial crab fishermen, thus making it possible the mature female drowned in a crab pot and washed ashore after being removed from a pot that was currently in use (Walters, unpublished). Extensive studies conducted in other estuarine ecosystems often find that commercial style crabpots have a catch rate of 0.027 to 0.49 terrapins pot^{-1} d^{-1} (Bishop 1983). Because the landscape genetic data (Chapter 2) indicates that open water is a feature that impedes gene flow of the terrapin and crab pots represent a specific threat to terrapins in open water, several actions should be taken in order to prevent deaths of males and females in crab pots. First, all crab pots should be required to have TEDs, not just those placed in waterways less than 150 feet wide since derelict pots can eventually end up in these areas (personal

observation, Figure 6-3). Second, surveys should be undertaken in Barnegat Bay to estimate their total impact on the terrapin population and to remove abandoned pots. Some states have adopted this measure. For example, in Chesapeake Bay, Virginia they removed 635 crab traps over a 33.5 km^2 survey using 600 kHz side scan sonar images (Havens *et al.* 2009).

Another particular threat to terrapins in the open water of Barnegat Bay, NJ is motor boats in Barnegat Bay. Incidences of injuries to the carapace or plastron of a terrapin due to boat strikes have been estimated to be between 1-4 % in North Forsythe and 5-11 % in South Forsythe from 2006-2009 (Lester, unpublished). It is presumed that boat propeller strikes can also kill terrapins on impact and thus injury rates likely underestimate the impact of motor boats on the terrapin population. Research is currently underway to determine the impact of boat encounters and boat engine sounds on the behavior of the terrapin in Barnegat Bay (Lester, unpublished and Harrison, unpublished). Because the landscape genetic data (Chapter 2) indicates that open water is a feature that impedes gene flow of the terrapin and that boats represent a threat to terrapins in the open water, management plans should aim to limit or reduce the use of motor boats and personal watercraft in waterways surrounding estuarine emergent wetland.

Road mortality is also plays a significant impact on the terrapin population in Barnegat Bay. In particular, at Great Bay Boulevard road mortality is known to cause deaths of nesting females (Hoden & Able 2003; Szerlag & McRobert 2006). In the 2004 nesting season, 600 nesting female occurrences were recorded and 53 nesting

females suffered road mortality (8.8%; Szerlag & McRobert 2006). Females often nest along roadsides after the destruction of historical nesting beaches because the sandy soils are the only suitable habitat available for nesting. Females continue to suffer road mortality at Great Bay Boulevard and along other roads, such as Cedar Run Dock Road and on Dock Road next to West Creek, in Barnegat Bay, NJ (personal observation). Roads leading to increased direct mortality are known to have several possible genetic consequences that can increase a species extinction probability (Balkenhol & Waits 2009). The genetic consequences can include: reduced effective population sizes, reduced gene flow, increased genetic structure, and decreased genetic diversity (Balkenhol & Waits 2009). Because landscape genetic data (Chapter 2) indicates that development is a feature that impedes gene flow of the terrapin and road mortality is a specific threat to female terrapins in upland areas that are developed, management plans should aim to prevent road mortality of females. These plans should include signs along the roadside to warn drivers that terrapins may be on the road, use of barriers to prevent females from entering the road, and the reestablishment of lost nesting habitats. Conservation plans that involve erecting roadside barriers should consider whether the barriers (although aimed to reduce mortality) will ultimately prevent gene flow. If natural corridors are no longer present (e.g. creeks passing under a bridge or the road), it may be necessary to create artificial corridors to maintain gene flow. Long-term research studies, utilizing multiple research approaches (e.g. molecular, telemetry, capture-mark-recapture) should be implemented to compare differences between areas with and

without roads to those areas in which conservation actions were aimed at mitigating the effects of roads (Balkenhol & Waits 2009).

The data presented in this dissertation demonstrate females are philopatric to natal beaches (Chapter 3) and consequently that beach protection should be addressed in conservation management plans. Although more work needs to be completed to determine how females would behaviorally respond to the loss of a natal nesting beach (research currently planned for the 2010 nesting season), nesting beaches should be protected in several ways. First, nesting beaches should be protected from conversion into developed areas or from blocked access due to bulkheading (Figure 6-4). Habitat restoration using dredge materials is being considered a potentially viable solution in areas where beaches have been destroyed by humans or natural erosion (U.S. Army Corps of Engineers 2008). However, studies have indicated lower rates of hatching success in dredged material when compared to natural sandy soils due to the high salt concentrations in dredge material influencing water potential of the soils (Wnek 2010). Beaches made from dredge materials have the potential to contain higher levels of persistent organic pollutants than natural nesting beaches and consequently may result in higher levels of transfer of these pollutants to eggs incubated in dredge soils. Persistent organic pollutants measured in terrapin tissues have been associated with disruptions in their physiological systems (Basile 2010). Thus, dredge materials may need to be properly treated prior to their use as material for nesting beach restoration. Second, human activities should be kept to a minimum on known nesting beaches and in the water surrounding the beach, particularly during

the nesting season. Increases in human activity on nesting beaches may cause terrapins to nest on beaches where less human disturbance occurs (Roosenburg 1994). Some turtles alter nesting behavior when disturbed or when they perceive danger (Iverson and Smith 1993; Spencer 2002; Spencer and Thompson 2003). Human activities could potentially affect both the decision of a female to emerge from the water to nest and habitat selection (Bowen & Janzen 2008). Turtles may also abandon nesting attempts as the result of direct human activities (Bowen & Janzen 2008). Currently, anecdotal information suggests nesting females in Barnegat Bay are less likely to nest on beaches when human activities are ongoing (John Wnek, personal communication). On the other hand, females may become habituated to human disturbance (Whittaker & Knight 1998). Even if human activities do not stop females from nesting, the actions of individual humans, such as removing nesting turtles via pet trade and road mortality, can still have detrimental efforts to a population (Bowen & Janzen 2008). Lastly, in addition to the protection of females, females' clutches should also be protected, especially in nesting areas where the both number of nesting females and hatching success rates are low. Protection of clutches from a large number of females will help to 1) reduce genetic correlations of individuals on a nesting beach and thus reduce the rate of loss of genetic diversity and 2) boost population sizes, especially those facing population declines as a result of human impacts.

The data presented in this dissertation also demonstrated that multiple paternity in the diamondback terrapin was influenced by the sex ratio of the

population (Chapter 4, Figure 4-2) and consequently anthropogenic alterations to population sex ratios should be minimized. Thus conservation management plans should include measures to eliminate threats that differentially affect males and females. These threats include: boat strikes (Cecala *et al.* 2008), vehicles strikes (Wood & Herlands 1997), and crab pot mortality (Roosenburg *et al.* 1997). Conservation management plans should also continually monitor the mating system and genetic diversity terrapin populations, especially in populations with extremely biased sex ratios and declining population sizes.

6.4 Directions for future research

This dissertation has answered many questions regarding the mating system, dispersal, and genetics of the terrapin, but throughout the course of this research, many new questions have arisen. The groundwork has now been laid for more complex and advanced questions that may expand on the work presented in this dissertation. Although not a complete list of research questions, the following list provides an example of the additional topics that could be explored:

1) *Will additional sampling locations throughout Barnegat Bay and New Jersey further resolve landscape genetic features affecting gene flow in the terrapin? Can the models be extended to predict the impacts of sea level rise due to global warming?*
2) *Can the landscape model in this study be extended to terrapins found in other areas throughout its range? In particular, will the model change*

based on estuary depth, tidal height, and other habitat features (e.g. mangrove vs. Spartina saltmarsh)?

3) *Why is estuarine emergent wetland responsible for shaping spatial genetic structure in the terrapin? For which life stage(s) of the terrapin is emergent wetland a critical habitat? This research should include analyses of physiology (e.g. osmoregulation and thermoregulation), feeding ecology, and dispersal ability of different sexes and size classes.*
4) *Female terrapins exhibit natal philopatry; if preferred nesting beaches are unavailable how do female terrapins behaviorally respond? This research could include tracking studies in which females are blocked access to beaches. Are there any consequences to the female's lifetime fitness?*
5) *When and how does imprinting of nesting beach occur? This could include tracking experiments where gravid females are transplanted away from the nesting beach where they were captured. It would also be useful to perform a longitudinal tracking study of females that are transplanted away from nesting beaches at different life stages (e.g. as embryos, hatchlings, or juveniles) to determine the stage at which natal nesting beach imprinting occurs.*
6) *How does population density in combination with sex ratio affect the frequency of multiple mating and sperm storage?*
7) *Do females from female biased populations utilize stored sperm more frequently? This research would require multiple years of obtaining nests*

from the same females for several years in populations with different sex ratios.

8) *What factors affect sperm competition in the terrapin? In particular, does the paternity (and paternity skew in multiple paternity clutches) accurately reflect the sperm stored in the oviduct? Are some sperm from males more numerous in the oviduct, but less successfully represented in the clutch? Is there any difference in morphology of the successful sperm? Does success of stored sperm change over time, even if females do not mate again?*
9) *Does sexual selection occur and if so what characteristics are males or females selecting? This will require an extensive sampling of successful males fathering females clutches.*
10) *Do terrapins form groups or social bonds? For example, are multi-year clutches in which the same father sired the offspring of a single female truly the result of stored sperm or are females mating with the same male multiple times over several years?*
11) *Are terrapins in Barnegat Bay capable of laying larger eggs? If so, what is the advantage to terrapins in the northern part of their range in laying smaller eggs with larger clutches rather than smaller clutches with larger eggs? In particular, it would be interesting to manipulate egg size of females from southern and northern populations and then randomly incubate and rear them in southern and northern climate conditions.*

Figure 6-1. A hoop trap with leaders placed in shallow water (<1 m deep) adjacent to estuarine emergent wetland.

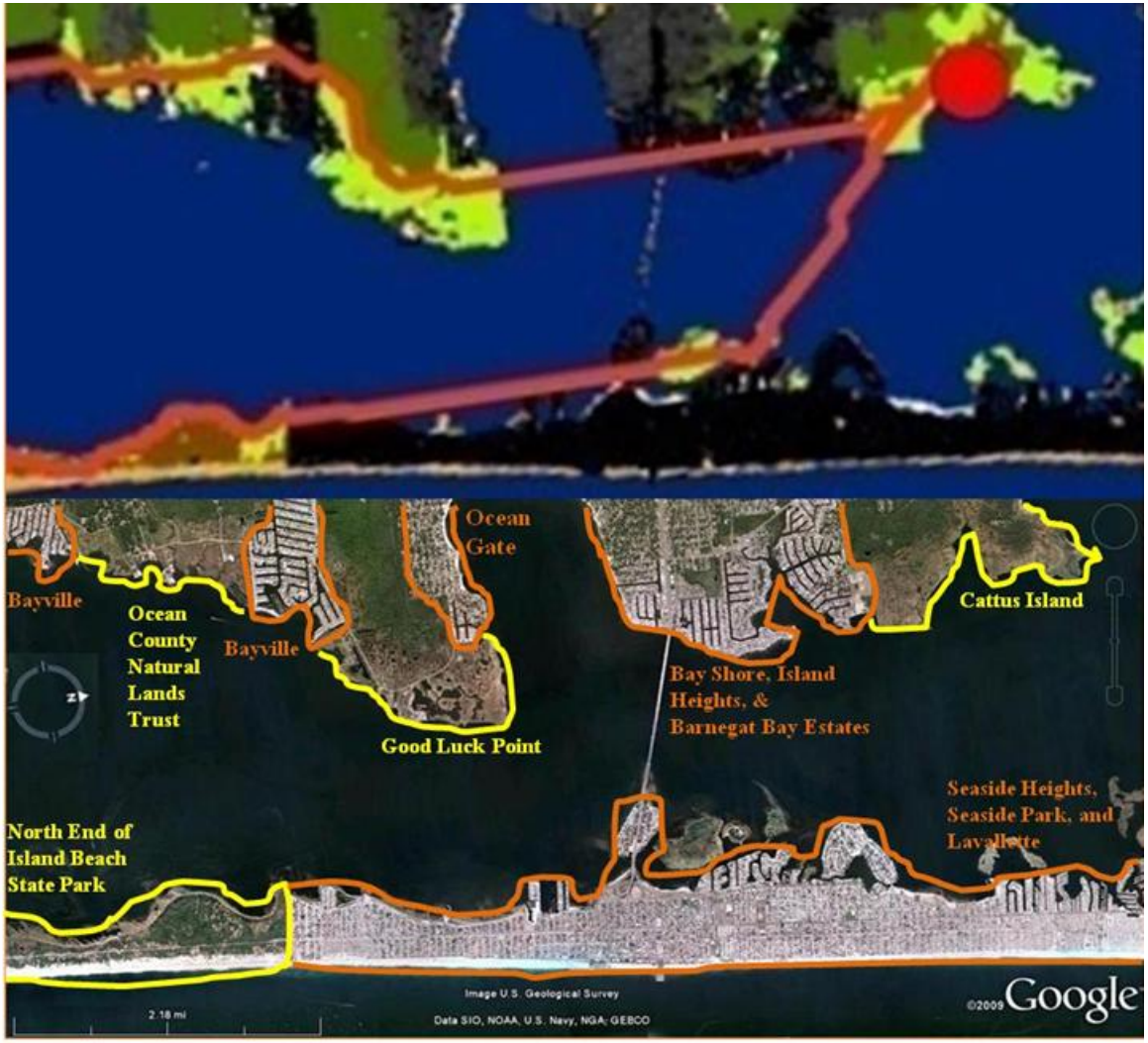


Figure 6-2. Example of the utilization of the genetic landscape model (top) to determine locations where further habitat degradation should be avoided and where habitat restoration may be beneficial to terrapin populations (bottom).

Figure 6-3. A male and female terrapin caught in a "ghost" crab pot recovered behind Conklin Island in North Forsythe during the summer of 2007. This crab pot did not have a terrapin excluder device (TED) at the time of recovery. The pot was found in approximately 0.5 m of standing water during low tide and would have been completely covered by standing water during high tide. This pot also captured a juvenile female.

Figure 6-4. Example of bulkheading along the shoreline in Barnegat Bay. Nesting females do not have the ability to access the sandy area behind the bulkheading for nesting. Photo credit: Dr. Harold Avery

LIST OF REFERENCES

Akaike H (1974) New look at statistical-model identification. *IEEE Transactions on Automatic Control* AC19, 716–723.

Alacs EF, Janzen F, Scribner KT (2007) Genetic issues in freshwater turtle and tortoise conservation. *Chelonian Conservation and Biology* 4, 107-123.

Allman PE (2006) Consequences of egg size on hatchling energetics in the diamondback terrapin, *Malaclemys terrapin*: a geographic comparison. Ph.D. dissertation, Ohio University, Athens, Ohio.

Anderson C, Epperson B, Fortin M-J, *et al.* (In Review) The importance of spatial and temporal scale in landscape genetics. *Molecular Ecology*.

Anderson DR, Burnham KP, White GC (2001) Kullback-Leibler information in resolving natural resource conflicts when definitive data exist. *Wildlife Society Bulletin* 29, 1260–1270.

Andren H (1995) Effects of habitat fragmentation on birds and mammals in landscapes with different proportions of suitable habitat: a review. *Oikos* 71, 355-366.

Anthony LL, Blumstein DT (2000) Integrating behaviour into wildlife conservation: the multiple ways that behaviour can reduce Ne. *Biological Conservation* 95, 303-315.

Arens P, van der Sluis T, van't Westende WPC, *et al.* (2007) Genetic population differentiation and connectivity among fragmented Moor frog (*Rana arvalis*) populations in the Netherlands. *Landscape Ecology* 22, 1489-1500.

Aresco MJ (1996) *Malaclemys terrapin terrapin* (Northern Diamondback terrapin) nesting and predation. *Herpetological Review* 27, 77.

Auger PJ (1989) Sex ratio and nesting behavior in a population of *Malaclemys terrapin* displaying temperature-dependent sex-determination. Masters Thesis, Tufts University, Medford, Massachusetts.

Avery HW, Vitt LJ (1984) How to get blood from a turtle. *Copeia* 1984, 209–210.

Avissar NG (2006) Changes in population structure of diamondback terrapins (*Malaclemys terrapin terrapin*) in a previously surveyed creek in southern New Jersey. *Chelonian Conservation and Biology* 5, 154-159.

Baldwin JD, Latino LA, Mealey BK, Parks GM, Forstner MRJ (2005) The diamondback terrapin in Florida Bay and the Florida Keys: insights into turtle conservation and ecology. In: *Amphibians and Reptiles: status and conservation in Florida* (eds. Meshaka WE, Babbitt KJ), pp. 180-186. Krieger Publishing Company, Malabar, Florida.

Balkenhol N, Waits LP (2009) Molecular road ecology: Exploring the potential of genetics for investigating transportation impacts on wildlife. *Molecular Ecology* **18**, 4151-4164.

Balloux F, Brunner H, Lugon-Moulin N, Hausser J, Goudet J (2000) Microsatellites can be misleading: An empirical and simulation study. *Evolution* 54, 1414-1422.

Bartlett JG, Mageean DM, O'Connor RJ (2000) Residential Expansion as a Continental Threat to U.S. Coastal Ecosystems. *Population and Environment* 21, 429-468.

Basile E (2010) Persistent organic pollutants in diamondback terrapin *(Malaclemys terrapin)* tissues and eggs and sediments from Barnegat Bay NJ. Ph.D. Dissertation, Drexel University, Philadelphia, Pennsylvania.

Bates D, Maechler M (2010) lme4: Linear mixed-effects models using S4 classes.

Baur BA (2004) Nesting Ecology of the northern diamondback terrapin (Order Testudines*; Malaclemys terrapin terrapin*). Masters Thesis, C.W. Post Long Island University, New York, USA.

BBNEP (2002) Barnegat Bay National Estuary Program: Comprehensive Conservation and Management Plan. < http://www.bbep.org/ccmp.html>

BBNEP (2003) Barnegat Bay National Estuary Program: Monitoring Program Plan. < http://www.bbep.org/dwnloads/Mon_Plan.pdf>

Beck NR, Peakall R, Heinsohn R (2008) Social constraint and an absence of sex-biased dispersal drive fine-scale genetic structure in white-winged choughs. *Molecular Ecology* 17, 4346-4358.

Beier P, Majka DR, Newell SL (2009) Uncertainty analysis of a least-cost modeling approach for designing wildlife linkages. *Ecological Applications* **19**, 2067-2077.

Beier P, Majka DR, Spencer WD (2008) Forks in the road: choices in procedures for designing wildland linkages. *Conservation Biology* **22**, 836-851.

Berven KA, Grudzien. TA (1990) Dispersal in the wood frog (*Rana sylvatica*): implications for genetic structure. *Evolution* 44, 2047-2056.

Birkhead TR, Moller AP (1993) Sexual selection and the temporal separation of reproductive events: sperm storage data from reptiles, birds and mammals. *Biological Journal of the Linnean Society* 50, 295-311.

Bishop JM (1983) Incidental capture of diamondback terrapins by crab pots. *Estuaries* 6, 426-430.

Bjørnstad ON, Andreassen HP, Ims RA (1998) Effects of habitat patchiness and connectivity on the spatial ecology of the root vole (*Microtus oeconomus*). *Journal of Animal Ecology* 67, 127-141.

Blanchong JA, Samuel MD, Scribner KT, *et al.* (2008) Landscape genetics and the spatial distribution of chronic wasting disease. *Biological Letters* 4, 130-133.

Bollmer JL, Irwin ME, Rieder JP, Parker PG (1999) Multiple paternity in loggerhead turtle clutches. *Copeia* 1999, 475-478.

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. *Trends in Ecology & Evolution* 13, 202-206.

Boudjemadi K, Lecomte J, Clobert J (1997) Influence of connectivity on demography and dispersal in two contrasting habitats: an experimental approach. *Journal of Animal Ecology* 68, 1207-1224.

Bowden RM, Harms HK, Paitz RT, Janzen FJ (2004) Does optimal egg size vary with demographic stage because of a physiological constraint? *Functional Ecology* 18, 522-529.

Bowen KD, Janzen FJ (2008) Human Recreation and the Nesting Ecology of a Freshwater Turtle (*Chrysemys picta*) *Chelonian Conservation and Biology* 7, 95-100.

Breden F (1987) The effect of post-metamorphic dispersal on the population genetic structure of Fowler's toad, *Bufo woodhousei fowleri*. *Copeia* 1987, 386-395.

Brennessel B (2006) *Diamonds in the Marsh: A natural history of the Diamondback terrapin* University Press of New England, Lebanon, New Hampshire.

Brockelmam WY (1975) Competition, the fitness of offspring, and optimal clutch size. *American Naturalist* 109, 677-699.

Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. *Molecular Ecology* 5, 453–455.

Buhlmann KA, Lynch TK, Gibbons JW, Greene JL (1995) Prolonged egg retention in the turtle *Deirochelys reticularia* in South Carolina. *Herpetologica* 51, 457-462.

Burger J (1977) Determinants of Hatching Success in Diamondback Terrapin, *Malaclemys Terrapin*. *American Midland Naturalist* 97, 444-464.

Burger J (2002) Metals in tissues of diamondback terrapin from New Jersey *Environmental Monitoring and Assessment* 77, 255-263.

Burger J, Montevecchi WA (1975) Nest site selection in the terrapin *Malaclemys terrapin*. *Copeia* 1975, 113-119.

Burnham KP, Anderson DR (2002) *Model selection and inference—a practical information-theoretic approach* Springer, New York. 353 p.

Burnham KP, Anderson DR, White GC, Brownie C, Pollock KH (1987) Design and analysis methods for fish survival experiments based on release-recapture. *American Fisheries Society Monograph* 5, 1-437.

Burns TA, Williams KL (1972) Notes on the reproductive habits of *Malaclemys terrapin pileata*. *Herpetology* 6, 237-238.

Butler JA (2000) Status and distribution of the Carolina diamondback terrapin, *Malaclemys terrapin centrata*, in Duval County. Florida Fish and Wildlife Conservation Commission Tallahassee, Florida.

Butler JA (unpublished) Population ecology, home range, and seasonal movements of the Carolina diamondback terrapin, *Malaclemys terrapin centrata*, in northeastern Florida. 2002 Final Report. Florida Fish and Wildlife Conservation Commission, Tallahassee, Florida, USA.

Butler JA, Broadhurst C, Green M, Mullin Z (2004) Nesting, nest predation, and hatchling emergence of the Carolina diamondback terrapin, *Malaclemys terrapin centrata*, in northeastern Florida. *American Midland Naturalist* 152, 145-155.

Cagle FR (1939) A system for marking turtles for future identification. *Copeia* 1939, 170-173.

Cagle FR (1954) Observations on the life cycles of painted turtles (Genus *Chrysemys*). *American Midland Naturalist* 52, 225-235.

Cagle FR, Tihen J (1948) Retention of Eggs by the Turtle *Deirochelys reticularia*. *Copeia* 1948, 66.

Cale PG (2003) The influence of social behaviour, dispersal and landscape fragmentation on population structure in a sedentary bird. *Biological Conservation* 109, 237–248.

Carr AF (1952) *Handbook of turtles* Comstock Publishing Associates, Ithaca, New York.

Castellano CM, Behler JL, Amato G (2009) Genetic diversity and population genetic structure of the Wood Turtle (*Glyptemys insculpta*) at Delaware Water Gap National Recreation Area, USA. *Conservation Genetics* 10, 1783-1788.

Cecala KK, Gibbons JW, Dorcas ME (2008) Ecological effects of major injuries in diamondback terrapins: implications for conservation and management. *Aquatic Conservation: Marine and Freshwater Ecosystems*. DOI: 10.1002/aqc.

Chakraborty R, De Andrade M, Daiger S, Budowle B (1992) Apparent heterozygote deficiencies observed in DNA typing data and their implications in forensic applications. *Annals of Human Genetics* 56, 45–57.

Chesser RK, Baker RJ (1996) Effective sizes and dynamics of uniparentally and diparentally inherited genes. *Genetics* 144, 1225-1235.

Christiansen JL, Moll EO (1973) Latitudinal reproductive variation within a single subspecies of painted turtle, *Chrysemys picta belii*. *Herpetologica* 29, 152-163.

Clark PJ, Ewert MA, Nelson CE (2001) Physical Apertures as Constraints on Egg Size and Shape in the Common Musk Turtle, Sternotherus odoratus. *Functional Ecology* 15, 70-77.

Clark RW, Brown WS, Stechert R, Zamudio KR (2007) Integrating individual behaviour and landscape genetics: the population structure of timber rattlesnake hibernacula *Molecular Ecology* 17, 719-730.

Cody ML (1966) A general theory of clutch size. *Evolution* 20, 174-184.

Coker RE (1906) The cultivation of the diamondback terrapin. *Bulletin of the North Carolina Geological Survey* 14, 1-69.

Conant R (1964) Turtle Soup. *America's First Zoo* 16.

Congdon JD (1989) Proximate and evolutionary constraints on energy relations of reptiles. *Physiological Zoology* 62, 356-373.

Congdon JD, Dunham AE, Van Loben Sels RC (1993) Delayed sexual maturity and demographics of blanding's turtles (*Emydoidea blandingii*): Implications for conservation and management of long-lived organisms. *Conservation Biology* 7, 826-833.

Congdon JD, Gibbons JW (1987) Morphological constraint on egg size: a challenge to optimal egg size theory? *Proceedings of the National Academy of Sciences* 84, 4145-4147.

Congdon JD, Gibbons JW, Greene JL (1983) Parental investment in the chicken turtle (*Deirochelys reticularia*). *Ecology* 64, 419-425.

Congdon JD, Greene JL, Gibbons JW (1986) Biomass of freshwater turtles: a geographic comparision. *American Midland Naturalist* 115, 165-173.

Congdon JD, Nagle RD, Kinney OM, Sels RCV (2003) Testing hypotheses of aging in long-lived painted turtles (*Chrysemys picta*). *Experimental Gerontology* 38, 765-772.

Congdon JD, Tinkle DEW (1982) Reproductive energetics of the painted turtle (*Chrysemys picta*). *Herpetologica* 38, 228-237.

Cooper MJP, Beever MD, Oppenheimer M (2005) Future sea level rise and the New Jersey coast: Assessing potential impacts and opportunities, p. 37. Woodrow Wilson School of Public and International Affairs, Princeton University, Princeton, NJ.

Crandell KA, Bininda-Emonds ORP, Mace GM, Wayne RK (2000) Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution* 15, 290-229.

Crim JL, Spotila LD, Spotila JR, *et al.* (2002) The leatherback turtle, *Dermochelys coriacea*, exhibits both polyandry and polygyny. *Molecular Ecology* 11, 2097-2106.

Crooks K, Sanjayan M (2006) *Connectivity Conservation: Maintaining Connections for Nature* Cambridge University Press, Cambridge, UK.

CRSSA (1999) Barnegat Bay Bulkheading The Grant F. Walton Center for Remote Sensing and Spatial Analysis (CRSSA), Rutgers University (original: U.S. Geological Survey) New Brunswick, New Jersey (original: Reston, Virginia)

CRSSA (2004a) 2001 Level 1 Landsat 7 ETM+ Satellite Image Land Cover Classification of New Jersey. Center for Remote Sensing and Spatial Analysis (CRSSA), Rutgers University New Brunswick, NJ.

CRSSA (2004b) Submerged aquatic vegetation (SAV) and bottom type CRSSA image classification within Barnegat Bay, Little Egg Harbor and Great Bay, New Jersey, Yr 2003. Center for Remote Sensing and Spatial Analysis (CRSSA), Rutgers University New Brunswick, New Jersey.

Culliton TJ, Warren MA, Goodspeed TR, *et al.* (1990) 50 years of population change along the nation's coasts 1960-2010. Second Report of a Coastal Trends Series. National Oceanic and Atmospheric Administration, Rockville, MD. 41 p.

Cushman SA, McKelvey KS, Hayden J, Schwartz MK (2006) Gene flow in complex landscapes: testing multiple hypotheses with causal modeling. *American Naturalist* 168, 486-489.

Daily GC (1995) Restoring value to the world's degraded lands. *Science* 269, 350-354.

Davenport J, Macedo EA (1990) Behavorial osmotic control in the euryhaline diamondback terrapin *Malaclemys terrapin*: trophic, spatial and temporal foraging constraints. *Journal of Zoology (London)* 220, 487-496.

Davenport J, Spikes M, Thorton SM, Kelly BO (1992) Crab-eating in the diamondback terrapin *Malaclemys terrapin*: dealing with dangerous prey. *Journal of the Marine Biological Association of the United Kingdom* 72, 825-848.

Díaz-Paniagua C, Keller C, Andreu AC (2001) Long-term demographic fluctuations of the spur-thighed tortoise *Testudo graeca* in SW Spain. *Ecography* 24, 707-721.

Dorcas ME, Willson JD, Gibbons JW (2007) Crab trapping causes population decline and demographic changes in diamondback terrapins over two decades. *Biological Conservation* 137, 334-340.

Double MC, Peakall R, Beck NR, Cockburn A (2005) Dispersal, philopatry, and infidelity: Dissecting local genetic structure in superb fairy-wrens (*Malurus cyaneus*). *Evolution* 59, 625-635.

Dubey S, Brown GP, Madsen T, Shine R (2008) Male-biased dispersal in a tropical Australian snake (*Stegonotus cucullatus*, Colubridae). *Molecular Ecology* 17, 3506-3514.

Dunson WA, Mazzotti FJ (1989) Salinity as a limiting factor in the distribution of reptiles in Florida Bay: a theory for the estuarine origin of marine snakes and turtles. *Bulletin of Marine Science* 44, 229-244.

Eberhard WG (1998) Female roles in sperm competition. In: *Sperm competition and sexual selection* (eds. Birkhead TR, Moller AP), pp. 91-116. Academic Press, San Diego, CA.

Echelle AA, Hackler JC, Lack JB, *et al.* (2009) Conservation genetics of the alligator snapping turtle: cytonuclear evidence of range-wide bottleneck effects and unusually pronounced geographic structure. *Conservation Genetics*.

Einum S, Fleming IA (2000) Highly fecund mothers sacrifice offspring survival to maximize fitness. *Nature* 405, 565-567.

Ellegren H (2000) Microsatellite mutations in the germline: implications for evolutionary inference. *Trends in Genetics* 16, 551-558.

Emaresi G, Pellet J, Dubey S, Hirzel AH, Fumagalli L (2009) Landscape genetics of the Alpine newt (*Mesotriton alpestris*) inferred from a strip-based approach. *Conservation Genetics,* 1566-0621.

Emlen ST, Oring LW (1977) Ecology, sexual selection, and the evolution of mating systems. *Science* 197, 215-223.

Epperson BK (1990) Spatial patterns of genetic variation within plant populations. In: *Population Genetics, Breeding and Genetic Resources* (eds. Brown AHD, Clegg MT, Kahler AL, Weir BS), pp. 229-253. Sinauer Assoc. Inc., Sunderland, Massachusetts.

Epperson BK, Li TQ (1996) Measurement of genetic structure within populations using Moran's spatial autocorrelation statistics. *Proceedings of the National Academy of Sciences of the United States of America* 93, 10528-10532.

Epps C, Wehausen JD, Bleich VC, Torres SG, Brashares JS (2007) Optimizing dispersal and corridor models using landscape genetics. *Journal of Applied Ecology* **44**, 714-724.

Ernst CH, Lovich JE, Barbour R (1994) *Turtles of the United States and Canada* Smithsonian Institution, Washington, D.C.

Escalona T, Engstrom TN, Hernandez OE, *et al.* (2009) Population genetics of the endangered South American freshwater turtle, *Podocnemis unifilis*, inferred from microsatellite DNA data. *Conservation Genetics* 10, 1683-1696.

Estep RL (2005) Seasonal movement and habitat use patterns of a diamondback terrapin (*Malaclemys terrapin*) population. Masters Thesis, The Graduate School of the College of Charleston.

Ewert MA, Legler JM (1978) Hormonal induction of oviposition in turtles. *Herpetologica* 34, 314-318.

Fahrig L (2003) Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology Evolution and Systematics* 34, 487-515.

Fahrig L, Merriam G (1994) Conservation of fragmented populations. *Conservation Biology* 8, 50-59.

Favre L, Balloux F, Goudet J, Perrin N (1997) Female-biased dispersal in the monogamous mammal *Crocidura russula*: evidence from field data and microsatellite patterns. *Proc. R. Soc. Lond. Ser. B* 264, 127–132.

Fitzsimmons NN (1998) Single paternity of clutches and sperm storage in the promiscuous green turtle (*Chelonia mydas*). *Molecular Ecology* 7, 575-584.

FitzSimmons NN, Hart KM (2007) Genetic studies of freshwater turtles and tortoises: a review of the past 70 years. *Chelonian Research Monographs* 4, 15-46.

Fiumera AC, Porter BA, Looney G, Asmussen MA, Avise JC (2004) Maximizing offspring production while maintaining genetic diversity in supplemental breeding programs of highly fecund managed species. *Conservation Biology* 18, 94–101.

Ford NB, Seigel RA (1989) Relationships among body size, clutch size, and egg size in three species of oviparous snakes. *Herpetologica* 45, 75-83.

Freedberg S, Ewert MA, Ridenhour BJ, Neiman M, Nelson CE (2005) Nesting fidelity and molecular evidence for natal homing in the freshwater turtle, Graptemys kohnii. *Proceedings of the Royal Society B-Biological Sciences* 272, 1345-1350.

Freedberg S, Wade MJ (2001) Cultural inheritance as a mechanism for population sex-ratio bias in reptiles. *Evolution* 55, 1049-1055.

Fretwell SD (1969) The adjustment of birth rate to mortality in birds. *Ibis* 111, 624-627.

Funk WC, Blouin MS, Corn PS, *et al.* (2005) Population structure of Columbia spotted frogs (*Rana luteiventris*) is strongly affected by the landscape. *Molecular Ecology* 14, 483-496.

Galbraith DA (1991) Studies of mating systems in wood turtles (*Clemmys insculpta*) and snapping turtles *(Chelydra serpentina)* using DNA fingerprinting. Ph.D. dissertation, Queen's University.

Galbraith DA, Graesser CJ, Brooks RJ (1988) Egg retention by a snapping turtle, *Chelydra serpentina*, in central Ontario. *Canadian Field-Naturalist* 102, 734.

Gibbons JW, Greene JL (1979) X-ray photography: a technique to determine reproductive patterns of freshwater turtles. *Herpetologica* 35, 86-89.

Gibbons JW, Lovich JE, Tucker AD, FitzSimmons NN, Greene JL (2001) Demographic and ecological factors affecting conservation and management of the diamondback terrapin (*Malaclemys terrapin*). *Chelonian Conservation and Biology* 4, 66-74.

Gibbons JW, Scott DE, Ryan TJ, *et al.* (2000) The global decline of reptiles, Deja Vu amphibians. *Bioscience* 50, 653-666.

Gill DE (1978) The metapopulation ecology of the red-spotted newt, *Notophthalmus viridescens* (Rafinesque). *Ecological Monographs* 48, 145-166.

Gist DH, Congdon JD (1998) Oviductal sperm storage as a reproductive tactic of turtles. *Journal of Experimental Zoology* 282, 526-534.

Gist DH, Jones JM (1989) Sperm storage within the oviduct of turtles. *Journal of Morphology* 199, 379-384.

Goodwin CC (1994) Aspects of nesting ecology of the diamondback terrapin (*Malaclemys terrapin*) in Rhode Island. Masters Thesis, University of Rhode Island, Kingston, Rhode Island.

Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. *Journal of Heredity* 86, 485-486.

Goudet J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3).

Goudet J, Perrin N, Waser P (2002) Tests for sex-biased dispersal using bi-parentally inherited genetic markers. *Molecular Ecology* 11, 1103-1114.

Handley LJL, Perrin N (2007) Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology* 16, 1559-1578.

Harden LA, Diluzio NA, Gibbons JW, Dorcas ME (2007) Spatial and thermal ecology of the diamondback terrapin (Malacelmys terrapin) in a South Carolina salt marsh. *Journal of the North Carolina Academy of Sciences* 123, 154-162.

Harry JL, Briscoe DA (1988) Multiple paternity in the loggerhead turtle (*Caretta caretta*). *Journal of Heredity* 79, 96-99.

Hart K (2005) Population biology of diamondback terrapins *(Malaclemys terrapin):* defining and reducing threats across their geographic range. Ph.D. dissertation, Duke University, Durham, North Carolina.

Hart KM, McIvor CC (2008) Demography and ecology of mangrove diamondback terrapins in a wilderness area of Everglades National Park, Floridia, USA. *Copeia* 2008, 200-208.

Hartl DL (1988) *A primer of population genetics* Sinauer Associates, Inc., Sunderland, Massachusetts.

Hartl DL, Clark GC (1997) *Principles of Population Genetics*, 3rd edn. Sinauer Associates, Sunderland, MA.

Hauswaldt JS (2004) Population genetics and mating pattern of the diamondback terrapin (*Malaclemys terrapin*). Ph.D. Dissertation, University of South Carolina, Columbia, South Carolina.

Hauswaldt JS, Glenn TC (2005) Population genetics of the diamondback terrapin (*Malaclemys terrapin*). *Molecular Ecology* 14, 723–732.

Havens KJ, Bilkovic DM, Stanhope D, Angstadt K (2009) Derelict Blue Crab Traps in the Virginia Portion of the Chesapeake Bay. In: *Center for Coastal Resources Management*. Virginia Institute of Marine Science.

Hedrick PW (2005) A standardized genetic differentiation measure. *Evolution* 59, 1633-1638.

Hendry AP, Day T, Cooper AB (2001) Optimal size and number of propagules: allowance for discrete stages and effects of maternal size on reproductive output and offspring fitness. *American Naturalist* 157, 387-407.

Herlands R, Wood R, Pritchard J, Clapp H, Le Furge N (2004) Diamondback terrapin (*Malaclemys terrapin*) head-starting project in southern New Jersey. In: *Conservation and Ecology of Turtles of the Mid-Atlantic Region: A Symposium* (eds. Swarth C, Roosenburg WM, Kiviat E), pp. 13-18. Biblomania, Salt Lake City, Utah.

Hernandez-Divers SM, Hernandez-Divers JS, Wyneken J (2002) Angiographic, anatomic, and clinical technique descriptions of a subcarapacial venipuncture site for chelonians. *Journal of Herpetological Medicine and Surgery* 12, 32-37.

Hildebrand SF (1928) Review of the experiments on artificial culture of the diamond-back terrapin. *Bulletin of the United States Bureau of Fisheries* 45, 25-70.

Hoden R, Able KW (2003) Habitat use and road mortality of diamondback terrapins (*Malaclemys terrapin*) in the Jacques Cousteau National Estuarine Research Reserve at Mullica River-Great Bay in southern New Jersey. Institute of Marine and Coastal Sciences, Rutgers, The State University of New Jersey New Brunswick, NJ, Jacques Cousteau NERR Technical Report100–24.

Hoekert WEJ, Neufeglise H, Schouten AD, Menken SBJ (2002) Multiple paternity and female-biased mutation at a microsatellite locus in the olive ridley sea turtle (*Lepidochelys olivacea*). *Heredity* 89, 107-113.

Hogan JL (2003) Occurrence of the Diamondback Terrapin (Malaclemys terrapin littoralis) at South Deer Island in Galveston Bay, Texas, April 2001–May 2002. USGS, Austin, Texas.

Hoske DJ, Blanckenhorn WU (1999) Female multiple mating inbreeding avoidance and fitness: it's not only the magnitude of costs and benefits that count. *Behavioral Ecology* 10, 462-464.

Hulin V, Delmas V, Girondot M, Godfrey M, Guillon J-M (2009) Temperature-dependent sex determination and global change: are some species at greater risk? *Oecologia* 160, 493-506.

Hurd LE, Smedes GW, Dean TA (1979) An ecological study of natural population of diamondback terrapins (*Malaclemys terrapin*) in a Delaware salt marsh. *Estuaries* 2, 228-233.

Hurvich CF, Tsai CL (1989) Regression and time series model selection in small samples. *Biometrika* 76, 297-307.

Iverson JB (1991) Life history and demography of the yellow mud turtle, *Kinosternon flavescens*. *Herpetologica* 47, 373-395.

Iverson JB (1992) *A revised checklist with distribution maps of the turtles of the world* Iverson Publishing, Richmond, Ind.

Iverson JB, Balgooyen CP, Byrd KK, Lyddan KK (1993) Latitudinal variation in egg and clutch size in turtles. *Canadian Journal of Zoology* 71, 2448-2461.

Jackson CG, Jr., Landry MM, Jackson MM (1971) Reproductive tract anomaly in a box turtle. *Journal of Wildlife Diseases* 7, 175-177.

Jensen MP, Abreu-Grobois FA, Frydenberg J, Loeschcke V (2006) Microsatellites provide insight into contrasting mating patterns in arribada vs. non-arribada olive ridley sea turtle rookeries. *Molecular Ecology* 15, 2567-2575.

Jeyasuria P, Roosenburg WM, Place AR (1994) Role of P450 aromatase in sex determination of the diamondback terrapin, *Malaclemys terrapin*. *Journal of Experimental Zoology* 270, 95-111.

Ji X, Dub W-G, Lia H, Lina L-H (2006) Experimentally reducing clutch size reveals a fixed upper limit to egg size in snakes, evidence from the kingsnake *Elaphe carinata*. *Comparative Biochemistry and Physiology Part A Molecular & Integrative Physiology* 144, 474-478.

Johnston EE, Rand MS, Zweifel SG (2006) Detection of multiple paternity and sperm storage in a captive colony of the central Asian tortoise, *Testudo horsfieldii*. *Canadian Journal of Zoology* 84, 520-526.

Jones AG (2005) GERUD 2.0: a computer program for the reconstruction of parental genotypes from half-sib progeny arrays with known or unknown parents *Molecular Ecology Notes* 5, 708-711.

Jones AG, Walker D, Lindström K, Kvarnemo C, Avise JC (2001) Surprising similarity of sneaking rates and genetic mating patterns in two populations of sand goby experiencing disparate sexual selection regimes. *Molecular Ecology* 10, 461-469.

Kamler JF, Ballard WB, Lemons PR, Mote K (2004) Variation in mating system and group structure in two populations of swift foxes, *Vulpes velox*. *Animal Behaviour* 68, 83-88.

Kana TW, Eiser WC, Baca BJ, Williams ML (1988) Chapter 3: New Jersey case study. In: *Greenhouse effect, sea level rise, and coastal wetlands* (ed. Titus JG). U.S. Environmental Protection Agency, Washington, D.C.

Keller LF, Waller DM (2002) Inbreeding effects in wild populations. *Trends in Ecology and Evolution* 17, 230-241.

Kichler K, Holder MT, Davis SK, Marques MR, Owens DW (1999) Detection of multiple paternity in the kemps ridley sea turtle with limited sampling. *Molecular Ecology* 8, 819-830.

King TL, Julian SE (2004) Conservation of microsatellite DNA flanking sequence across 13 emydid genera assayed with novel bog turtle (*Glyptemys muhlenbergii*) loci. *Conservation Genetics* 5, 719-725.

Kinneary JJ (2008) Observation on terrestrial behavior and growth in diamondback terrapin (*Malaclemys*) and snapping turtle (*Chelydra*) hatchlings. *Chelonian Conservation and Biology* 7, 118-119.

Kratochvil L, Frynta D (2006) Egg shape and size allometry in geckos (Squamata: Gekkota), lizards with contrasting egg shell structure: why lay spherical eggs? . *Journal of Zoology Systematics & Evolutionary Research*.

Lamb T, Avise JC (1992) Molecular and population genetic aspects of mitochondrial DNA variability in the diamondback terrapin, *Malaclemys terrapin*. *Journal of Heredity* 83, 262-269.

Lande R, Barrowclough GF (1987) Effective population size, genetic variation, and their use in population management In: *Viable Populations for Conservation* (ed. Soule ME), pp. 97-123. Cambridge University Press, Cambridge, U.K.

Lathrop Jr. RG, Love A (2007) Vulnerability of New Jersey's Coastal Habitats to Sea Level Rise. Grant F. Walton Center for Remote Sensing & Spatial Analysis, Rutgers University, New Brunswick, NJ, and American Littoral Society, Highlands, NJ, 17 pp. <http://crssa.rutgers.edu/projects/coastal/sealevel/>

Lathrop RG, J.A. Bognar, A.C. Hendrickson, and P.D. Bowers (1999) Data Synthesis Effort for the Barnegat Bay Estuary Program: Habitat Loss and Alteration in the Barnegat Bay Region. Center for Remote Sensing & Spatial Analysis, Technical Report, Rutgers University, New Brunswick, NJ. <http://www.crssa.rutgers.edu/projects/runj/datasnth.html>

Laurance F, Bierregaard R (1997) Tropical Forest Remnants: Ecology, Management, and Conservation of Fragmented Communities University of Chicago Press, Chicago, Illinois.

Lawson Handley LJ, Perrin N (2007) Advances in our understanding of mammalian sex-biased dispersal. *Molecular Ecology* 16, 1159-1578.

Lee AM (2003) Reproductive biology and seasonal testosterone patterns of the diamondback terrapin, *Malaclemys terrapin*, in the estuaries of Charleston, South Carolina. Masters Thesis, College of Charleston.

Lee PLM, Hays GC (2004) Polyandry in a marine turtle: Females make the best of a bad job. *Proceedings of the National Academy of Sciences of the United States of America* 101, 6530-6535.

Lee-Yaw JA, Davidson A, McRae BH, Green DM (2009) Do landscape processes predict phylogeographic patterns in the wood frog? *Molecular Ecology* 18, 1863-1874.

Lester LA (2007) Tracking terrapins through genetic analysis: multilocus assignment tests shed light on origin of turtles sold in markets. Masters Thesis, Duke University.

Levesque EM (2000) Distrubution and ecology of the diamondback terrapin (*Malaclemys terrapin*) in South Carolina salt marshes. Masters Thesis, College of Charleston, Charleston, South Carolina.

Long DR, Rose FL (1989) Pelvic girdle size relationships in three turtle species. *Journal of Herpetology* 23, 315-318.

Lovich JE (1996) Possible demographic and ecologic consequences of sex ratio manipulation in turtles. *Chelonian Conservation and Biology* 2, 114-117.

Lovich JE, Gibbons JW (1990) Age at maturity influences adult sex ratio in the turtle. *Oikos* 59.

Lowe A, Harris S, Ashton P (2004) *Ecological Genetics: Design, Analysis, and Application* Blackwell Publishing, Oxford, U.K.

Lynch M, Lande R (1998) The critical effective size for a genetically secure population. *Animal Conservation* 1, 70-72.

Manel S, Gaggiotti OE, Waples RS (2005) Assignment methods: matching biological questions techniques with appropriate. *Trends in Ecology & Evolution* 20, 136-142.

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. *Trends in Ecology & Evolution* 18, 189–197.

McGinley MA, Temme DH, Geber MA (1987) Parental investment in offspring in variable environments: theoretical and empirical considerations. *American Naturalist* 130, 370-398.

McLusky DS, Elliot M (2004) *The Estuarine Ecosystem: Ecology, Threats, and Management* Oxford University Press, Oxford, New York.

McRae BH (2006) Isolation by resistance. *Evolution* 60, 1551-1561.

McTaggert SJ (2000) Good genes or sexy sons? Testing the benefits of female mate choice in the painted turtle, *Chrysemys picta.* Masters Thesis, University of Guelph.

Meirmans PG (2006) Using the AMOVA framework to estimate a standardised genetic differentiation measure. *Evolution* 60, 2399-2402.

Miller L (1932) Notes on the desert tortoise (*Testudo agassizii*). *Transactions of the San Diego Society of Natural History* 7, 187-208.

Mitro M (2003) Demography and viability analyses of a diamondback terrapin population. *Canadian Journal of Zoology* 81, 716-726.

Mobley KB, Jones AG (2007) Geographical variation in the mating system of the dusky pipefish (*Syngnathus floridae*). *Molecular Ecology* 16, 2596-2606.

Mockford SW, McEachern L, Herman TB, Snyder M, Wright JM (2005) Population genetic structure of a disjunct population of Blandings turtle (*Emydoidea blandingii)* in Nova Scotia, Canada. *Biological Conservation* 123, 373–380.

Moll EO (1973) Latitudinal and intersubspecific variation in reproduction of the painted turtle, *Chrysemys picta*. *Herpetologica* 29, 307-318.

Moll EO, Legler JM (1971) The life history of a neotropicalkk slider turtle, *Pseudemys scripta* (Schoepff), in Panama. *Bulletin of the Los Angeles Museum of Natural History* 11, 1-102.

Montevecchi WA, Burger J (1975) Aspects of the Reproductive Biology of the Northern Diamondback Terrapin *Malaclemys terrapin terrapin*. *American Midland Naturalist* 94, 166-178.

Moon JC, McCoy ED, Mushinsky HR, Karl SA (2006) Multiple paternity and breeding system in the gopher tortoise, *Gopherus polyphemus*. *Journal of Heredity* 97, 150-157.

Moore JA, Miller HC, Daugherty CH, Nelson NJ (2008) Fine-scale genetic structure of a long-lived reptile reflects recent habitat modification. *Molecular Ecology* 17, 4630-4641.

Moore MK, Ball RM (2002) Multiple paternity in loggerhead turtle (*Caretta caretta*) nests on Melbourne Beach, Florida: a microsatellite analysis. *Molecular Ecology* 11, 281-288.

Morreale SJ, Gibbons JW, Congdon JD (1984) Significance of activity and movement in the yellow-bellied slider turtle (*Pseudemys scripta*). *Canadian Journal of Zoology* 62, 1038-1042.

Mushinsky HR, Wilson DS, McCoy ED (1994) Growth and Sexual Dimorphism of *Gopherus polyphemus* in Central Florida. *Herpetologica* 50, 119-128.

N.J.A.C. (1981) Environmental Protection Division of Fish and Wildlife: Fish and Game Council: Division of Fish and Wildlife Rules: Terrapin Regulations: 7:25-21.

Neff BD, Pitcher TE, Repka J (2002) A Bayesian model for assessing the frequency of multiple mating in nature. *Journal of Heredity* 93, 406–414.

Neff BD, Repka J, Gross MR (2001) A Bayesian framework for parentage analysis: the value of genetic and other biological data. *Theoretical Population Biology* 59, 315–331.

Nei M (1987) *Molecular Evolutionary Genetics* Columbia University Press, New York.

Ner SE (2003) Distribution and predation of diamondback terrapin nests at six upland islands of Jamaica Bay and Sandy Hook Unit, Gateway National Recreation Area. Masters Thesis, Hofstra University, Hempstead, New York.

Neville HM, Isaak DJ, Dunham JB, Thurow RF, Rieman BE (2006) Fine-scale natal homing and localized movement as shaped by sex and spawning habitat in Chinook salmon: insights from spatial autocorrelation analysis of individual genotypes. *Molecular Ecology* 15, 4589-4602.

NJDEP, OIRM, BGIS (2003a) NJDEP TIGER Roads 2000 in Atlantic County, New Jersey NJ Department of Environmental Protection (NJDEP), Office of Information Resources Management (OIRM), Bureau of Geographic Information Systems (BGIS) Trenton, NJ

NJDEP, OIRM, BGIS (2003b) NJDEP TIGER Roads 2000 in Ocean County, New Jersey NJ Department of Environmental Protection (NJDEP), Office of Information Resources Management (OIRM), Bureau of Geographic Information Systems (BGIS) Trenton, NJ

Olsson M, Gullberg A, Tegelström H (1994) Sperm competition in the sand lizard, *Lacerta agilis*. *Animal Behaviour* 48, 193–200.

Olsson M, Madsen T, Shine R, Gullberg A, Tegelström H (1994) Rewards of promiscuity. *Nature* 372, 230.

Olsson M, Shine R, Madsen T (1996) Sperm selection by females. *Nature* 383, 585.

Oosterhout CV, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes* 4, 535 - 538.

Paetkau D, Calvert W, Stirling I, Strobeck C (1995) Microsatellite analysis of population structure in Canadian polar bears. *Molecular Ecology* 4, 347-354.

Paetkau D, Slade R, Burden M, Estoup A (2004) Genetic assignment methods for the direct, real-time estimation of migration rate: a simulation-based exploration of accuracy and power. *Molecular Ecology* 13, 55-65.

Palmer KS, Rostal DC, Grumbles JS, Mulvey M (1998) Long-term sperm storage in the desert tortoise (*Gopherus agassizii*). *Copeia*, 702-705.

Parker PG, Waite TA, Peare T (1996) Paternity studies in animal populations. In: *Molecular genetic approaches in animal conservation* (eds. Smith TB, Wayne RK), pp. 413-423. Oxford University Press, New York.

Parker WS (1984) Immigration and dispersal of slider turtles *Pseudemys scripta* in Mississippi farm ponds. *American Midland Naturalist* 112, 280-293.

Peacock MM, Smith AT (1997a) The effect of habitat fragmentation on dispersal, mating behavior, and genetic variation in a pika (*Ochotona princeps*) metapopulation. *Oecologia* 112, 524-533.

Peacock MM, Smith AT (1997b) Non-random mating in pikas (*Ochotona princeps*): evidence for inbreeding between individuals of intermediate relatedness. *Molecular Ecology* 6, 801-812.

Peakall R, Ruibal M, Lindenmayer DB (2003) Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fuscipes*. *Evolution* 57, 1182-1195.

Peakall R, Smouse PE (2006) GENALEX 6: genetic analysis in Excel. Population software for teaching and research. *Molecular Ecology Notes* 6, 288-295.

Pearse DE, Anderson EC (2009) Multiple paternity increases effective population size. *Molecular Ecology* 18, 3124-3127.

Pearse DE, Avise JC (2001) Turtle mating systems: Behavior, sperm storage, and genetic paternity. *Journal of Heredity* 92, 206-211.

Pearse DE, Janzen FJ, Avise JC (2002) Multiple paternity, sperm storage, and reproductive success of female and male painted turtles (*Chrysemys picta*) in nature. *Behavioral Ecology and Sociobiology* 51, 164-171.

Perez-Espona S, Perez-Barberia FJ, McLeod JE, *et al.* (2008) Landscape features affect gene flow of Scottish Highland red deer (*Cervus elaphus*). *Molecular Ecology* 17, 981-996.

Petrie M, Kempenares B (1998) Extra-pair paternity in birds: explaining variation between species and populations. *Trends in Ecology and Evolution* 13, 52-58.

Petrochic SL (2009) Feeding ecology of the northern diamondback terrapin, *Malaclemys terrapin terrapin.* Masters Thesis, C. W. Post University of Long Island University, Brookville, New York

Piertney SB, MacColl ADC, Bacon PJ, Dallas JF (1998) Local genetic structure in red grouse (*Lagopus lagopus scoticus*): evidence from microsatellite DNA markers. *Molecular Ecology* 7, 1645-54.

Piry S, Alapetite A, Cornuet JM, *et al.* (2004) GENECLASS2: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity* 95, 536-539.

Powell CB (1967) Female sexual cycles of *Chrysemys picta* and *Clemmys insculpta* in Nova Scotia. *Canadian Field-Naturalist* 81, 134-140.

Pradel R (1993) Flexibility in survival analysis from recapture-data: handling trap-dependence. In: *Marked Individuals in the Study of Bird Populations* (eds. Lebreton JD, North PM). Birkhäuser Verlag Basel.

Pradel R, Hines JE, Lebreton JD, Nichols JD (1997) Capture-recapture survival models taking account of transients. *Biometrics* 53, 88-89.

Price PW (1974) Strategies for egg production. *Evolution* 28, 76-84.

Prugha LR, Hodges KE, Sinclair ARE, Brashares JS (2008) Effect of habitat area and isolation on fragmented animal populations. *Proceedings of the National Academy of Sciences of the United States of America* 105, 20770-20775.

Prugnolle F, de Meeus T (2002) Inferring sex-biased dispersal from population genetic tools: a review. *Heredity* 88, 161-165.

Pulsipher A, Pulsipher LM (2005) *World Regional Geography (Without Subregions): Global Patterns, Local Lives* Macmillan, United States.

Pusey A (1987) Sex-biased dispersal and inbreeding avoidance in birds and mammals. *Trends in Ecology and Evolution* 2, 295–299.

Queller DC, Goodnight KF (1989) Estimating relatedness using genetic markers. *Evolution* 43, 258-275

Quinn NWS, Tate DP (1991) Seasonal Movements and Habitat of Wood Turtles (*Clemmys insculpta*) in Algonquin Park, Canada. *Journal of Herpetology* 25, 217-220.

R Development Core Team (2010) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Raymond M, Rousset F (1995) An exact test for population differentiation *Evolution* 55, 1703-1705.

Reid GK, Jr. (1955) Reproduction and development in the northern diamondback terrapin, *Malaclemys terrapin terrapin*. *Copeia* 1955, 310-311.

Reinhold K (1998) Nest-site philopatry and selection for environmental sex determination. *Evolutionary Ecology* 12, 245-250.

Rhodin AGJ, Parham JF, van DIjk PP, Iverson JB (2009) Turtles of the World: Annotated Checklist of Taxonomy and Synonymy, 2009 Update, with Conservation Status Summary. *Chelonian Research Monographs* **5**, 39-84.

Rice WR (1989) Analyzing tables of statistical tests. *Evolution* 43, 223-225.

Rife A (2007) Social and basking behaviors in juvenile, captive-raised northern diamondback terrapins (*Malaclemys terrapin terrapin*). Undergraduate Thesis, Boston College.

Risley PL (1933) Observations on the natural history of the common musk turtle, *Sternotherus odoratus*. *Papers of the Michigan Academy of Science, Arts and Letters* 17, 685-711.

Robinson KM, Dunson WA (1976) Water and sodium balance in the estuarine diamondback terrapin (*Malaclemys*). *Journal of Comparative Physiology* 105, 129-152.

Roff DA (1992) *The evolution of life histories: theory and analysis* Chapman and Hall, New York.

Rollinson N, Brooks RJ (2008) Optimal offspring provisioning when egg size is "constrained": a case study with the painted turtle *Chrysemys picta*. *Oikos* 117, 144-151.

Roosenburg WM (1990) The Diamondback Terrapin: Population Dynamics, Habitat Requirements, and Opportunities for Conservation. In: *New Perspectives in the Chesapeake System: A Research and Management Partnership*, pp. 229-234. Chesapeake Research Consortium, Baltimore, MD.

Roosenburg WM (1994) Nesting habitat requirements of the diamondback terrapin: a geographic comparison. *Wetlands Journal* **6**, 9-12.

Roosenburg WM (1996) Maternal condition and nest site choice: an alternative for the maintenance of environmental sex determination? *American Zoologist* 36, 157-168.

Roosenburg WM (2004) The impact of crab pot fisheries on terrapin (*Malaclemys terrapin*) populations: where are we and where do we need to go? *Conservation and Ecology of Turtles of the Mid-Atlantic Region: A Symposium*, 23-30.

Roosenburg WM, Cover J, van Dijk PP (2008) Legal Issues: Legislative closure of the Maryland terrapin fishery: Perspecitives on a historical accomplishment. *Turtle and Tortoise Newsletter* 12, 27-30.

Roosenburg WM, Cresko W, Modesitte M, Robbins MB (1997) Diamondback terrapin (*Malaclemys terrapin*) mortality in crab pots. *Conservation Biology* 5, 1166-1172.

Roosenburg WM, Dennis T (2005) Egg component comparisions within and among clutches of the diamondback terrapin, *Malaclemys terrapin Copeia* 2005, 417-423.

Roosenburg WM, Dunham AE (1997) Allocation of reproductive output: egg- and clutch-size variation in the Diamondback terrapin. *Copeia* 1997, 290-297.

Roosenburg WM, Graham LJ, Heckman ML (2009) Terrapin Monitoring at Poplar Island, 2007, p. 69. Army Corps of Engineers, Baltimore Office, Baltimore, Maryland.

Roosenburg WM, Graham LJ, Matthews E (2007) Terrapin Monitoring at Poplar Island, 2006, p. 48. Army Corps of Engineers, Baltimore Office, Baltimore, Maryland.

Roosenburg WM, Haley KL, McGuire S (1999) Habitat selection and movements of diamondback terrapins, *Malaclemys terrapin*, in a Maryland estuary. *Chelonian Conservation and Biology* 3, 425-429.

Roosenburg WM, Kelley KC (1996) The effect of egg size and incubation temperature on growth in the turtle, *Malaclemys terrapin*. *Journal of Herpetology* 30, 198-204.

Roosenburg WM, Sullivan S (2006) Terrapin Monitoring at Poplar Island, 2005, p. 65. Army Corps of Engineers, Baltimore Office, Baltimore, Maryland.

Roques S, Diaz-Paniagua C, Andreu AC (2004) Microsatellite markers reveal multiple paternity and sperm storage in the Mediterranean spur thighed tortoise, *Testudo graeca*. *Canadian Journal of Zoology* 82, 153-159.

Roques S, Diaz-Paniagua C, Portheault A, Perez-Santigosa N, Hidalgo-Vila J (2006) Sperm storage and low incidence of multiple paternity in the European pond turtle, *Emys orbicularis*: A secure but costly strategy? *Biological Conservation* 129, 236-243.

Rose FL, Simpson TR, Manning RW (1996) Measured and Predicted Egg Volume of Pseudemys texana with Comments on Turtle Egg Shape. *Journal of Herpetology* 30, 433-435.

Ross KG (2001) Molecular ecology of social behavior: analyses of breeding systems and genetic structure. *Molecular Ecology* 10, 265-284.

Rountree RA, Smith KJ, Able KW (1992) Length frequency data for fishes and turtles from polyhaline subtidal and intertidal marsh creeks in southern New Jersey. Rutgers University, Institute of Marine and Coastal Sciences.

Royall RM (1997) *Statistical evidence: a likelihood paradigm* Chapman and Hall, New York.

Ryman N (2006) CHIFISH - a computer program testing for genetic heterogeneity at multiple loci using chi-square and Fisher's exact test. *Molecular Ecology Notes* 6, 285-287.

Saccheri IJ, Kuussaari M, Kankare M, *et al.* (1998) Inbreeding and extinction in a butterfly metapopulation. *Nature* 392, 491-494.

Sarre S, Schwaner TD, Georges A (1990) Genetic variation among insular populations of the sleepy lizard, *Trachydosaurus rugosus* (Squamata: Scincidae). *Australian Journal of Zoology* 38, 603-616.

Scavia D, Field JC, Boesch DF, *et al.* (2002) Climate change impacts on U.S. Coastal and Marine Ecosystems. *Estuaries and Coasts* 25, 149-164.

Schultz DL (1991) Parental invest in temporally varying environments. *Evolutionary Ecology* 5, 415-427.

Scribner KT, Blanchong JA, Bruggeman DJ, *et al.* (2005) Geographical genetics: conceptual foundations and empirical applications of spatial genetic data in wildlife management. *Journal of Wildlife Management* 69, 1434–1453.

Scribner KT, Chesser RK (2001) Group-structured models in analyses of the population and behavioral ecology of poikilothermic vertebrates. *Journal of Heredity* 98, 180-189.

Scribner KT, Congdon JD, Chesser RK, Smith. MH (1993) Annual differences in female reproductive success affect spatial and cohort-specific genotypic heterogeneity in painted turtles. *Evolution* 47, 1360-1373.

Scribner KT, Evans J, Morreale SJ, Smith MH, Gibbons JW (1986) Genetic variability among populations of the yellow-bellied slider turtle from aquatically and terrestrially separated habitats. *Copeia* 1986, 691-700.

Seigel RA (1979) The reproductive biology of the diamondback terrapin, *Malaclemys terrapin tequesta.* Masters Thesis, The University of Central Florida.

Seigel RA (1980) Nesting habits of diamondback terrapins (*Malaclemys terrapin*) on the Atlantic coast of Florida. *Transactions of the Kansas Academy of Science* 83, 239-246.

Seigel RA (1983) Apparent long term declines in diamondback terrapin populations at the Kennedy Space Centre, Florida. *Herpetological Review* 24, 102-103.

Seigel RA (1984) Parameters of two populations of diamondback terrapins (*Malaclemys terrapin*) on the Atlantic coast of Florida. p. 77-87. In: *Vertebrate ecology and systematics- a tribute to Henry S. Fitch* (eds. Seigel RA, Hunt LE, Knight JL, Malaret L, Zuschlag NL). University of Kansas, Lawrence

Seigel RA, Gibbons JW (1995) Workshop on the ecology, status, and management of the diamondback terrapin (*Malaclemys terrapin*) Savannah River Ecology Laboratory, 2 August 1994: final results and recommendations. *Chelonian Conservation and Biology* 1, 241-243.

Semlitsch RD (2000) Principles for management of aquatic breeding amphibians. *Journal of Wildlife Management* 64, 615-631.

Semlitsch RD, Bodie JR (2003) Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology* 17, 1219-1228.

Silliman BR, Bertness MD (2002) A trophic cascade regulates salt marsh primary production. *Proceedings of the National Academy of Sciences of the United States of America* 99, 10500-10505.

Sinervo B (1990) The evolution of maternal investment in lizards: An experimental and comparative analysis of egg size and its effects on offspring performance. *Evolution* 44, 279-294.

Sinervo B, Doughty P, Huey RB, Zamudio K (1992) Allometric engineering: a causal analysis of natural selection on offspring size. *Science* 258, 1927-1930.

Sinervo B, Licht P (1991a) Hormonal and physiological control of clutch size, egg size, and egg shape in side-blotched lizards (*Uta stansburiana*): constraints on the evolution of lizard life histories. *Journal of Experimental Zoology* 257, 252-264.

Sinervo B, Licht P (1991b) Proximate constraints on the evolution of egg size, number, and total clutch mass in lizards. *Science* 252.

Slatkin M (1985) Gene flow in natural populations. *Annual Review of Ecology and Systematics* 16, 393-430.

Smith CC, Fretwell SD (1974) The optimal balance between size and number of offpspring. *American Naturalist* 108, 499-506.

Smouse PE, Long JC, Sokal RR (1986) Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology* 35, 627-632.

Smouse PE, Peakall R (1999) Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity* 82, 561-573.

Soucy S, Travis J (2003) Multiple paternity and population genetic structure in natural populations of the poeciliid fish, *Heterandria formosa*. *Journal of Evolutionary Biology* 16, 1328-1336.

Souza FL, Cunha AF, Oliveira MA, Pereira GAG, dos Reis SF (2002) Estimating dispersal and gene flow in the neotropical freshwater turtle *Hydromedusa maximiliani* (Chelidae) by combining ecological and genetic methods. *Genetics and Molecular Biology* 25, 151-155.

Spear S, Balkenhol N, Fortin M-J, McRae B, Scribner K (In Review) Use of resistance surfaces for landscape genetic studies: Considerations for parameterization and analysis. *Molecular Ecology*.

Spear SF, Peterson CR, Matocq MD, Storfer A (2005) Landscape genetics of the blotched tiger salamander (*Ambystoma tigrinum melanostictum*). *Molecular Ecology* 14, 2553-2564.

Spear SF, Storfer A (2008) Landscape genetic structure of coastal tailed frogs (*Ascaphus truei*) in protected vs. managed forests. *Molecular Ecology* 17, 4642-4656.

Spivey PB (1998) Home range, habitat selection, and diet of the diamondback terrapin (*Malaclemys terrapin*) in a North Carolina estuary. Masters Thesis, University of Georgia, Athens, Georgia.

Stephens PA, Sutherland WJ (1999) Consequences of the Allee effect for behaviour, ecology and conservation. *Trends in Ecology & Evolution* 14, 401-405.

Stephens PR, Wiens JJ (2003) Ecological diversification and phylogeny of emydid turtles. *Biological Journal of the Linnean Society* 79, 577-610.

Stevens VM, Verkenne C, Vandewoestijne S, Wesselingh RA, Baguette M (2006) Gene flow and functional connectivity in the natterjack toad. *Molecular Ecology* 15, 2333-2344.

Storfer A, Murphy MA, Evans JS, *et al.* (2007) Putting the 'landscape' in landscape genetics. *Heredity* 998, 128-142.

Stow AJ, Sunnucks P (2004a) High mate and site fidelity in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. *Molecular Ecology* 13, 419-430.

Stow AJ, Sunnucks P (2004b) Inbreeding avoidance in Cunningham's skinks (*Egernia cunninghami*) in natural and fragmented habitat. *Molecular Ecology* 13, 443-447.

Stow AJ, Sunnucks P, Briscoe DA, Gardner MG (2001) The impact of habitat fragmentation on dispersal of Cunningham's skink (*Egernia cunninghami*): evidence from allelic and genotypic analyses of microsatellites. *Molecular Ecology* 10, 867-878.

Strange EM, Jones AS, Bosch C, *et al.* (2008) Mid-Atlantic Coastal Habitats and Environmental Implications of Sea Level Rise. Section 3. In: *Background Documents Supporting Climate Change Science Program Synthesis and Assessment Product 4.1* (eds. Titus JG, Strange EM). EPA. U.S., Washington, DC.

Sugg DW, Chesser RK (1994) Effective population sizes with multiple paternity. *Genetics* 137, 1147-1155.

Szerlag S, McRobert SP (2006) Road occurrence and mortality of the northern diamondback terrapin. *Applied Herpetology* 3, 27-37.

Szerlag S, McRobert SP (2007) Northern diamondback terrapin occurrence, movement, and nesting activity along a salt marsh access road. *Chelonian Conservation and Biology* 6, 295-301.

Temple HJ, Hoffman JI, Amos W (2006) Dispersal, philopatry and intergroup relatedness: fine-scale genetic structure in the white-breasted thrasher, *Ramphocinclus brachyurus*. *Molecular Ecology* 15, 3449-3458.

Tinkle DW (1961) Geographic variation in reproduction size, sex ratio and maturity of *Sternothaerus ordoratus* (Testudinata: Chelydridae). *Ecology* 42, 68-76.

Tregenza T, Wedell N (2000) Genetic compatibility, mate choice, and patterns of parentage. *Molecular Ecology* 9, 1013-1027.

Tucker AD, Fitzsimmons NN, Gibbons JW (1995) Resource partitioning by the estuarine turtle *Malaclemys terrapin*: trophic, spatial and temporal foraging constraints. *Herpetologica* 51, 167-181.

Tucker AD, Gibbons JW, Greene JL (2001) Estimates of adult survival and migration for diamondback terrapins: conservation insight from local extirpation within a metapopulation. *Canadian Journal of Zoology* 79, 2199-2209.

U.S. Army Corps of Engineers (2008) Ecosystem Restoration Project at Poplar Island: A National Model for Benefical Use of Dredged Material In: *US Army Corps of Engineers, Baltimore District*, Baltimore, Maryland.

United States Army Corps of Engineers (2003) Barnegat Bay Ecosystem Restoration Feasibility Report and Integrated Environmental Assessment (ed. New Jersey Department of Environmental Protection). <http://www.nap.usace.army.mil/Projects/Barnegat/index.htm>

US Department of Homeland Security, US Coast Guard, Office of Auxillary and Boating Safety (2009) Recreational Boating Statisitics 2008, Washington, DC. <http://www.uscgboating.org/assets/1/Publications/Boating_Statistics_2008.pdf>

Vitousek PM, Ehrlich PR, Ehrlich AH, Matson PA (1986) Human appropriation of the products of photosynthesis. *BioScience* 36, 368-373.

Vitousek PM, Mooney HA, Lubchenco J, Melillo JM (1997) Human domination of earth's ecosystems. *Science* 277, 494-499.

Waits LP, Luikart G, Taberlet P (2001) Estimating the probability of identity among genotypes in natural populations: cautions and guidelines. *Molecular Ecology* 10, 249-256.

Walker H (1990) The coastal zone. In: *The Earth as Transformed by Human Action: Global and Regional Changes in the Biosphere Over the Past 300 Years* (eds. Turner BL, Clark WC, Kates RW, *et al.*), pp. 271 -294. Cambridge University Press, Cambridge, UK.

Walther G-R, Post E, Convey P, *et al.* (2002) Ecological responses to recent climate change. *Nature* 416, 389–395.

Wang IJ (2009) Fine-scale population structure in a desert amphibian: landscape genetics of the black toad (*Bufo exsul*). *Molecular Ecology* 18, 3847-3856.

Wang IJ, Savage WK, Shaffer HB (2009) Landscape genetics and least-cost path analysis reveal unexpected dispersal routes in the California tiger salamander (*Ambystoma californiense*). *Molecular Ecology* 18, 1365–1374

Watters CF (2004) A review of the range-wide regulations pertaining to diamondback terrapins (*Malaclemys terrapin*), pp. 1-12. Wetlands Institute, Stone Harbor, NJ.

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. *Evolution* 38, 1358-1370.

Westneat DF, Sherman PW (1997) Density and extra-pair fertilizations in birds: a comparative analysis. *Behavioral Ecology and Sociobiology* 41, 205-215.

Whitcomb R, CS R, Lynch J, *et al.* (1981) Effects of forest fragmentation on avifauna of the eastern deciduous forest. In: *Forest island dynamics in man-dominated landscapes* (eds. Burgess R, Sharpe B), pp. 125-206. Springer-Verlag, New York.

White GC, Burnham KP (1999) Program MARK: Survival estimation from populations of marked animals. *Bird Study 46 Supplement*, 120-138.

Whitlock MC, McCauley DE (1999) Indirect measures of gene flow and migration: FST not equal to 1/(4Nm+1). *Heredity* 82, 117-125.

Whittaker D, Knight RL (1998) Understanding wildlife responses to humans. *Wildlife Society Bulletin* **26**, 312-317.

Wilbur HM (1975) The evolutionary and mathematical demography of the turtle *Chrysemys picta. Ecology* 56, 64-77

Wilcox B, Murphy D (1985) Conservation strategy: the effects of fragmentation on extinction. *American Naturalist* 125, 879-887.

Wilkinson LR, Gibbons JW (2005) Patterns of reproductive allocation: clutch and egg size variation in three freshwater turtles. *Copeia* 2005, 868-879.

With K, Gardner R, Turner M (1997) Landscape connectivity and population distributions in heterogeneous environments. *Oikos* 78, 151-169.

Wnek J (2010) Anthropogenic impacts on the reproductive ecology of a model estuarine vertebrate, the diamondback terrapin, *Malaclemys terrapin.* Ph.D. Dissertation, Drexel University, Philadelphia, Pennsylvania.

Wood RC (1997) The impact of commercial crab traps on northern diamondback terrapins, *Malaclemys terrapin terrapin*. In: *Conservation, Restoration, and Management of Tortoises and Turtles--An International Conference* (ed. Abbema JV), pp. 21-27. New York Turtle and Tortoise Society, New York, USA.

Wood RC, Herlands R (1997) Turtles and tires: the impact of road kills on northern diamondback terrapin, *Malaclemys terrapin terrapin*, populations on the Cape May peninsula, southern New Jersey. In: *Conservation, Restoration, and Management of Tortoises and Turtles--An International Conference* (ed. Abbema JV), pp. 46-53. New York Turtle and Tortoise Society, New York, USA.

Wright S (1931) Evolution in Mendelian populations. *Genetics* 16, 97-159.

Wright S (1978) *Evolution and the Genetics of Populations.* University of Chicago Press, Chicago.

Yearicks EF, Wood RC, Johnson S (1981) Hibernation of the northern diamondback terrapin, *Malaclemys terrapin terrapin*. *Estuaries* 4, 78-80.

Young AG, Brown A, Murray BG, Thrall PH, Miller CH (2000) Genetic Erosion, Restricted Mating, and Reduced Viability in Fragmented Populations of the Endangered Grassland Herb *Rutidosis leptorrhynchoides*. In: *Genetics, Demography, and Viability of Fragmented Populations* (eds. Young AG, Clarke GM), pp. 335-360. Cambridge University Press, Cambridge.

Zimmerman TD (1989) Latitudinal reproductive variation of the salt marsh turtle, the diamondback terrapin (*Malaclemys terrapin*). Masters Thesis, University of Charleston, Charleston, South Carolina.

Curriculum Vita

Claire Marie Sheridan
Specialization: Conservation Biology, Molecular Ecology, Evolutionary Ecology,
Major Advisors: Harold W. Avery, Ph.D. and James R. Spotila, Ph.D.
Drexel University, Department of Biology
3141 Chestnut St., Philadelphia, PA 19104
Email: clairemarie63@yahoo.com

Education

Ph.D., Environmental Science/Ecology, 2005-2010: Drexel University, Philadelphia, PA.
B.A., Biology/Chemistry, 2005: Immaculata University, Immaculata, PA.

Research and Teaching Experience

2005-2008 **Field Team Leader/Doctoral Student**
Diamondback Terrapins of Barnegat Bay –Harold W. Avery, Ph.D., Principal Investigator

2003-2004 **Molecular Genetics Research Coordinator**- Coastal Conservation Research Program
Richard Stockton College and the Wetlands Institute – Peter Straub, Ph.D., Research Director

2005-2010 **Teaching Assistant, Drexel University,** Physiology and Nutrition, Cells and Genetics, Organismal Biodiversity and Ecology, Applied Biology, Environmental Science and Society

Publications: Peer Reviewed Literature

1. Sheridan CM, Spotila JR, Roosenburg WM, Bien WF, Avery HW. Inter-population variation of multiple paternity in the diamondback terrapin (*Malaclemys terrapin*). *Molecular Ecology*. In review.
2. Sheridan CM, Spotila JR, Bien WF, Avery HW. Sex-biased dispersal and natal philopatry in the diamondback terrapin, *Malaclemys terrapin*. *Molecular Ecology*. In review.
3. Sheridan CM, Scribner KT, Spotila JR, Bien WF, Avery HW. Landscape genetic structure in a highly fragmented ecosystem. In preparation. Target Journal: *PLOS Biology*
4. Sheridan CM, Wnek J, Spotila JR, Bien WF, Avery HW. Constraints on egg size, optimal egg size theory, and latitudinal reproductive variation in the diamondback terrapin (*Malaclemys terrapin).* In preparation. Target Journal: *Oikos*

Oral Presentations at Symposia

Habitat Fragmentation in the Barnegat Bay Estuary: Mating and Dispersal of the Northern Diamondback Terrapin(Malaclemys terrapin terrapin). Atlantic Estuarine Research Society: Clash of the Populations: Emerging Challenges for Coastal Lagoons. March 6, 2009.

The Dispersal of the Diamondback Terrapin in Relation to Habitat Fragmentation in the Barnegat Bay Estuary New Jersey. Evolution 2009, University of Idaho, Joint annual meeting of the Society for the Study of Evolution, the Society of Systematic Biologists, and the American Society of Naturalists. June 14, 2009.

Grants and Awards

Student Travel Grant, Graduate Studies, Office of the Provost, Drexel University 2009

Drexel University's Research Day 2009. Honorable Mention in Biological and Biomedical Research: Poster Presentation