Diamondback Terrapin, Malaclemys terrapin, Nesting and Overwintering Ecology

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This thesis titled

Diamondback Terrapin, Malaclemys terrapin, Nesting and Overwintering Ecology

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ABSTRACT

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Poplar Island Environmental Restoration Project is a unique solution for the dredge material placement and restoring decreasing habitat in the Chesapeake Bay. Since 2002, a long-term terrapin monitoring program has been documenting diamondback terrapin, *Malaclemys terrapin* habitat use. Northern diamondback terrapins, hatchlings may either emerge from their nest in the fall and seek other overwintering hibernacula, or remain inside their natal nest to emerge the following spring, known as delayed emergence. Results from the 2007-08 nesting season found that compaction and the presence of ice nucleating agents (as a measure of crystallization temperature) affected nest emergence timing in hatchlings. Fall emerged nests had lower bulk density (less compacted) and had a higher potency of ice nucleating agents compared to spring emerging nests. With proper management, areas such as Poplar Island may become areas of concentration for terrapins and thus provide a source population for the terrapin recovery throughout the Bay.

Approved: _____

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CHAPTER 1: TERRAPIN NESTING ECOLOGY

Increases in human populations result in; habitat destruction, habitat infrastructure deterioration, introduced species, subsidized predators, and overexploitation of natural resources for food and pets (Klemens, 2000). Estuarine ecosystems continue to be threatened as human population growth increases in coastal areas and development increases habitat loss, shoreline erosion, and subsidence (Mitro, 2003). These combined with global climate change's effect on sea levels results in loss of shoreline habitat and suitable terrapin nesting habitat in the Chesapeake bay (CENAB, 2009a). The United States' coastal ecosystems act as a storm buffers for communities, purify waters, and sustain coastal economies with billions of dollars in fisheries, tourism, transportation, and recreational income (Costra-Pierce and Weinstein, 2002). However, as states experience increase in population growth and development, habitat loss and the degradation of water quality threaten coastal economies (Costra-Pierce and Weinstein, 2002). As encroachment continues, local, state, and the federal government are turning to restoration to recreate habitat (Klemens, 2000). The Clean Water Action Plan and the Coastal Wetlands Protection, Planning, and Restoration Act are working to increase the area of restored wetland in the US (Costra-Pierce and Weinstein, 2002). Disposal of uncontaminated dredge materials into the Nation's waters and landfills creates an unnecessary waste of America's ecological, economic, engineering and scientific wealth (Costra-Pierce and Weinstein, 2002). Coastal wetland and beach ecosystem restoration has been identified as a national priority by the National Research Council and

potentially offers the opportunity to use uncontaminated dredge material in a constructive manner. (Costra-Pierce and Weinstein, 2002).

The Chesapeake Bay is the largest estuary in North America, covering 500 hectares of water (USACE, 2006) with a 16,575,900 hectare watershed. The Chesapeake Bay has over 3,600 species of flora and fauna in this complex ecosystem with a human population that exceeds 18 million people (CBP, 2009). The bay was created over 10,000 years ago with the retreat of the last glaciations in the Susquhanna River Valley. The Algonquins, Native Americans, residing in the bay watershed, called the bay Chesepiook, meaning "great shellfish bay". Today the bay provides millions of dollars in commercial and recreational value from its recreational and commercial fishery industry. The commercial and recreational species include blue crab, oyster, striped bass, and waterfowl. The bay also provides economic and educational resources in a multiuser environment (USACE, 2006). The protection and restoration of the bay's resources is considered vital to its future (USACE, 2006).

The port of Baltimore also is vital to the region's commerce. The Baltimore Port is one of the busiest ports on the East Coast (USACE, 2006; CENAB, 2009b). Its inland location and access to highways give it the ability to access manufacturing centers in the Midwest and one third of all U.S. households in a day's drive (USACE, 2006). The Baltimore Port Authority handles over 40 million tons of cargo annually, and foreign commerce valued at \$26 billion (USACE, 2006). The Baltimore Port contributes \$1.9 billion in business to Maryland's economy and generates over 50,000 jobs (CENAB, 2009b). In order to keep the port navigable, dredging of the waterways and canals leading to the port is necessary. In the next twenty years, however, there will be critical shortage of placement capacity and sites for dredged material from the Baltimore Harbor and its approach channels (USACE, 2006).

Under the USACE Engineering Regulation (ER) 1105-2-100, the USACE Districts must develop Dredged Material Management Plans for all federally maintained harbors and waterways. The plans must address the placement of dredge material with minimal environmental impact and identify projects that provide sufficient placement capacity to accommodate maintenance dredging (USACE, 2006). One USACE solution for dredged material from Chesapeake & Delaware Approach Canals and Chesapeake Bay Approach Canals (MD) was to create environmental restoration islands (Figure 1). Poplar Island Environmental Restoration Project (PIERP) is a unique man made island that restores upland and wetland habitat that is being lost throughout the bay area. I explore how diamondback terrapins, *Malaclemys terrapin*, use nesting beach habitat that has been created by PIERP. Most research on wildlife habitat use on dredge material islands has been focused on the avifauna or benthic populations and communities. Poplar Island Environmental Restoration Project has monitored terrapin nesting activity since 2002 and herein I document the terrapin nesting there to identify how large-scale restoration projects affect terrapin populations.

Materials and Methods

Study Site: Poplar Island Environmental Restoration Project

Poplar Island is an environmental restoration project in the middle Chesapeake Bay at 38°46' N and 76°23' W, approximately 34 nautical miles southeast of Baltimore

and 1 mile northwest of Tilghman Island, MD (USACE, 2006 CENAB, 2009a). The U.S. Army Corps of Engineers, Maryland Port Administration, and Maryland Environmental Services are reconstructing Poplar Island using dredged material from the Chesapeake and Delaware Canal approach channels and the Chesapeake Bay approach channels. The island is being restored to its original size of 400 hectares in the 1800s after having been eroded to less than 4 hectares by 1998 (CENAB, 2009a). Stone perimeter dikes prevent erosion of the island and protect exposed shores; interior and sheltered dikes are constructed of sand. The PIERP goal is to provide long term stable storage dredge material while simultaneously creating upland and wetland habitats that existed in the Poplar Island Archipelago 150 years ago. The wetland cells will constitute more than 297 hectacres of the island's area with restoration hydrodynamics, vegetation, and wildlife characteristic of the Chesapeake Bay salt marsh ecosystem (CENAB, 2009a; CENAB, 2009b; USACE, 2006). Construction of the island began in 1998 and completion is expected by 2027 (USACE, 2006). Plans are to use approximately 38 million cubic yards of uncontaminated dredge material (Dalal and Baker, 1999). In 1997, a Project Cooperation Agreement was executed with the State of Maryland with the project to be cost-shared 75 percent federal and 25 percent non-federal with the current project cost estimated at approximately \$667 million (CENAB, 2009b; USACE, 2006).

Poplar Island is isolated and human activity restricted to allow wildlife colonization and expansion in the archipelago (CENAB, 2009b). Additionally, the removal of foxes and raccoons, dominant terrapin nest predators, creates an ideal environment. Therefore, Poplar Island Environmental Restoration Project is unique because major predators are absent, which allows for a large detailed study of terrapin nesting ecology and how their populations respond to newly formed habitat via either natural or anthropogenic means. On Poplar Island, diamondback terrapin nests are found primarily along the east side on sandy strips (Cell 3, the notch, Cell 5,) and a few along the inside perimeter of Cell 6. These elevated nesting areas were built from sand that was mined from the sight.

Study Species: Diamondback terrapin, Malaclemys terrapin

The only turtle in North America that lives exclusively in estuaries is the diamondback terrapin, *Malaclemys terrapin* (Klemens, 2000). There are only two other exclusively estuarine turtles are *Batagur baska* and *Callagur borneoensis*, both found in Asia in tropical climates. The terrapin has one of the greatest geographic distributions for a turtle and may be found in a variety of habitats throughout their range (Roosenburg, 1994). Seven subspecies are found from Cape Cod, Massachusetts to Corpus Chrisi, Texas (Ernst et al., 1994). The northern diamondback terrapin is found from Cape Cod, Massachusetts to Cape Hatteras, North Carolina (Ernst et al., 1994). Diamondback terrapins evolved in coastal habitats and with the retreat of the last glaciations, expanded their range northward and inland (Roosenburg, 1994).

While terrapins require a whole suite of habitats to complete their lifecycle, they spend most of their life in water and come ashore to nest (Roosenburg, 1991; Roosenburg, 1994). Terrapins are found in salt marshes, tidal creeks, estuaries, and lagoons that lie behind barrier islands (Ehret and Werner, 2004; Roosenburg, 1991). However, terrapins in Florida are primarily found in lagoons (Roosenburg, 1994), while terrapins in New Jersey, Delaware and Maryland are found in channels and salt marshes (Roosenburg, 1994). In order to successfully reproduce, terrapins must cross the intertidal zone and place their nests above the mean high tide line (Roosenburg and Place, 1995).

Throughout their range, terrapins nest in a variety of habitats above the high water mark (Roosenburg, 1994; Roosenburg et al., 2003). In the Chesapeake, inland populations nest in open sandy patches above the mean high water. Coastal populations nest on large sand dunes that offer open sandy habitats (Roosenburg and Dunham, 1997; Roosenburg, 1994). Diamondback terrapins are iteroparous, nesting as many as three times during the nesting season (Roosenburg and Dunham, 1997). They are also philopatric, nesting on the same beach within and among years (Roosenburg, 1991). Terrapins will utilize suitable habitat when it is available because they are opportunists (Roosenburg, 1991). Terrapins dig small flask shaped chambers and deposit an average of 13 eggs in the Chesapeake Bay region. Terrapins exhibit temperature-dependent-sexdetermination (Roosenburg and Place, 1995).

Terrapins play an important role in estuaries. Terrapins feed primarily on filter feeders including soft and hard shell clams, razor clams, oysters, mussels, and barnacles (Bauer, 2004). These filter feeders consume plankton and zooplankton. Terrapins also consume browsers and detritivores such as whelks, marine worms, several species of crabs, and intertidal snails (Bauer, 2004). Terrapins prey on periwinkle snails which feed off of fungi that grow on salt marsh stems. If left unchecked, periwinkle snails will overgraze on and kill salt marsh grasses(Silliman and Bertness, 2002). Therefore, terrapins are potentially a keystone predator because they directly affect snail densities, distribution, abundance and diversity of the salt marsh community (Silliman and Bertness, 2002).

Terrapins are preyed on by a whole variety of predators throughout their lifecycle. Nests are preved upon by beach grass as roots grow into eggs (Lazell and Auger, 1981; Stegmann et al., 1988), fungi (Auger and Giovannone 1979), flies (Auger and Giovannone 1979), birds (Larus sp.) (Watkins-Colwell and Black 1997), ghost crabs (Ocypode quadrata) (Zimmerman, 1992), raccoons (Seigel, 1980), and foxes. Hatchlings' predators are fish, birds (Larus sp.) (Watkins-Colwell and Black 1997), raccoons (Procyon lotor) (Seigel, 1980), Roosenburg and Place, 1995), and foxes (Vulpes vulpes) (Burger, 1977). Adults are preved upon by raccoons and bald eagles (Clark, 1982). Raccoons are predators of all age classes of *M. terrapin*, including adult females. Raccoons catch and kill adult females while they're nesting to get their eggs (Roosenburg personal communication). Raccoons are a highly generalized nocturnal predator found in the eastern half of North America (Klemens, 2000). They are predators of eggs, hatchlings, adults or some combination for at least 58% of North American turtles and are considered the single most significant predator of turtles in North America (Klemens, 2000; Ernst et al., 1994). High densities of predators present increase mortality rates in turtle populations (Klemens, 2000). Roosenburg observed nest predation rates at two beaches on the Patuxent River in Maryland from 1987 to 1991, to average 83.5% at high density nesting beaches and 41.3% at a low density beaches. Predation at the first beach reached 95% in 1987 and 1988. Raccoons were the major nest predators (Roosenburg, 1991).



Figure 1. Poplar Island and dredge material location from Chesapeake and Delaware Approach Canals in the Chesapeake Bay (NASA, 2008).



Figure 2. Diamondback terrapin nesting habitats on Poplar Island; Cell 3 beach, the "notch", Cell 5 beach, and the inside perimeter of Cell 6 (Ariel photo CENABa, 2009 courtesy W.M.R.).

Poplar Island Field Methods

Diamondback terrapins began to nest on Poplar Island after the completion of the perimeter dike in 2002 (Roosenburg and Allman, 2003). Terrapin surveys taken from 2004 – to present have been consistent and detailed. Survey techniques and methods used for 2004-2007 nesting seasons are described in detail (Roosenburg et al., 2004, 2007, 2009; Roosenburg and Sulllivan 2006) and described herein briefly.

Daily surveys of terrapin nesting areas occurred from May 15 - August 1, 2007 in the following areas: the notch area (near Cell 4), areas between Coaches Island and the PIERP (outside of Cell 5), inside the open upland cell (Cell 6), and the beach outside the dike in Poplar Harbor (outside Cell 3) (Figure 2). Subtle changes in ground cover and terrapin tracks were used to locate nests. Once found, recent nests less than 24 hours old (indicated by the eggs' pink appearance) were excavated, weighed, and counted to obtain clutch size and egg mass. Eggs were then returned to original nest chamber and covered. Nests older than 24 hours (indicated by eggs' white appearance) were not excavated to prevent damage to the embryo. Geographic positioning system (GPS) recorded all nest positions. Beginning in 2006, nests were covered with antipredator 30 cm by 30 cm, 1.25 cm² wire mesh screens that were held in place by 4 survey flags. Screens were used to deter avian nest predators, primarily crows.

Monitoring nesting and hatching success:

After 45 - 50 days of incubation individual nests were encircled with an aluminum flashing ring to catch hatchlings and a 1.25 cm² wire mesh was placed over the ring to prevent avian predation. Once ringed, nests were checked daily for newly

emerged hatchlings. The hatchlings were then taken to an on-site laboratory facility where they were measured (carapace, plastron, width, and height), notched (marked marginal scutes for the cohort year), and tagged (a coded wire tag) (Roosenburg and Allman, 2003). Nests in Cell 5 and the notch, that did not have any hatchlings emerge in the fall, were left to overwinter with aluminum flashing ring and antipredator cage. All other nests in Cell 6, and Cell 3 were excavated by October 31.

Ten days after emergence of the last hatchling, researchers excavated nests and recorded the number of live hatchlings, dead hatchlings that remained, eggs with dead embryos, and eggs that showed no signs of development. Hatching success was determined by comparing the number of surviving hatchlings to the total number of eggs from only the nests that were excavated at ovipoisition. Nests that over-wintered were excavated early spring to determine fate of nests.

Measuring, tagging, and release of hatchlings:

All hatchlings were brought to the Maryland Environmental Service (MES) shed onsite and were placed in plastic containers with water until they were processed (measured, notched, and tagged) within 24 hours of capture (Roosenburg et al., 2009). Hatchlings were marked by marginal scute notching with a scalpel with a unique series for each cohort. Coded wire tags (CWTs, Northwest marine Technologies) were implanted in all hatchlings. The CWTs were placed subcutaneously in the right rear limb using a 25-gauge needle. The CWTs allow for long-term identification of the turtle by detecting tag presence or absence using Northwest Marine Technologies V-Detector. Plastron length, carapace length, width and height ($\pm 0.1 \text{ mm}$) and mass $\pm 0.1 \text{ g}$) were measured on all hatchlings. Anomalous scute patterns and other developmental irregularities were recorded. Hatchlings were released in Cell 4DX or Cell 3D. Institutional Animal Care and Uses Committee at Ohio University (IACUC) approved animal use protocols (#L01-04) and Maryland Department of Natural Resources (MD DNR) Fisheries Division issued a Scientific Collecting Permits to Willem M. Roosenburg (WMR).

Statistical Analysis

Significance of statistical analyses was accepted at P < 0.05. Data were processed using Microsoft Excel and Sigma Plot and statistical analyses were conducted using Statistical Analysis Systems (SAS) and R.

Results

Terrapins use available and accessible nesting areas on Poplar Island since 2002 (Figure 2). Nesting occurs along the beach of Cell 3, Cell 5, the "notch", and inside the perimeter of Cell 6 (Figure 2). The densest nesting occurs opposite Coaches Island in Cell 5 and along the notch (Figure 3). The number of nests in each major nesting site on PIERP has changed throughout the study (Figure 4). The number of nests along Cell 5 have increased and the number of nests along the notch have decreased from 2005-2007 (Figure 4). The proportion of nests surviving in each nesting area is consistent among years (Figure 4).

The total number of nests on Poplar Island have increased since the beginning of the monitoring program from 68 in 2002 to a peak of 282 nests in 2005 (Table 1).

Recently about 200 nests are found every year. Depredation increased from 2005 and 2006 then decreased in 2007 (Table 1).

Nests are only allowed to overwinter along the notch and Cell 5 due to logistics of monitoring all nesting areas throughout the year. All other nests are excavated in late October at which terrapin nest fate was determined and recorded. Looking at nest fate and overwintering percentage between 2006 and 2007 in the notch and Cell 5, the nests destroyed before fall emergence decreased from 2006 to 2007, hence the number of fall emerging nests increased in 2007 (Table 2). The proportion of nests that overwinter on Poplar Island along the notch and Cell 5 is about 30% each year (Table 2). There was one nest in 2006 that had both fall and spring emerging hatchlings.

There was a lay date effect on lipid levels in hatchlings in 2005, where nests laid later in the season had higher energy reserves than nests laid earlier in the season (ANOVA, $F_{1,28} = 7.65$, P < 0.01). There was no difference in lipid mass between fall and spring emerging hatchlings and lay date does not appear to affect emergence time (Figure 5).

There is no difference in the mean within nest survivorship (the proportion of eggs that were laid verses the number of hatchlings that were produced) between fall and spring emerging nests, from 2004-2007 (ANOVA, $F_{1,406}$ =2.75, p>0.05; Figure 6). There was a year effect (ANOVA, $F_{3,406}$ =8.63, p <0.05; Figure 6) with the lowest survivorship in 2005. There was no year by season interaction (ANOVA, $F_{3,406}$ = 1.7, p>0.05; Figure 6).

There is no effect of lay date on emergence time (fall or spring) in 2005 and 2007. A Wilcoxon rank sum test with continuity correction was used for 2005 and 2007 (2005: W = 1464.5, p-value > 0.5; N=128 fall emerged nests, N= 23 spring emerged nests, 2007: W = 2933, p-value > 0.5; N= 108 fall emerged nests, N=50 spring emerged nests; Figure 7). However there was a lay date effect in 2006 where nests laid early in the season emerged in the fall compared to nests laid later in the season, which emerged in the spring Wilcoxon rank sum test with continuity correction (W = 694, p-value < 0.05; N=62 fall emerged nests, N=30 spring emerged nests; Figure 7)



Figure 3. Terrapin nesting locations from 2002-2007. More recent years are on the bottom, overlapped by earlier nesting season years. (Ariel photo CENABa, 2009 courtesy W.M.R.).



Figure 4. The number of nests in each of the major nesting areas for each year of the study and the proportion of nests surviving.

Table 1

Poplar Island Terrapin Nest Fate 2002-2007

Year	2002	2003	2004	2005	2006	2007
Total nests	68	67	182	282	191	225
Nests produced hatchlings	38	50	129	176	112	166
Nests that did not survive	1	7	17	70	69	44
Depredated (roots or animal)	0	0	12	46	54	18
Washed out	1	6	3	11	13	2
Undeveloped, weak shelled eggs, or dead embryos	0	1	0	12	1	19
Destroyed by a turtle or nest was in rocks	0	0	0	1	0	0
Destroyed by bulldozer	0	0	0	0	1	2
Dead hatchlings	29	10	36	36	10	19

Table 2

Nest Fate and Overwintering Percentage

Year	2006	% 2006	2007	% 2007
Total Nests (notch and Cell 5)	146		170	
Depredated nests and nests destroyed before fall emergence	47	32.2%	18	10.6%
Fall emerging nests	49	33.6%	92	54.1%
Nests overwintering	44	30.1%	60	35.3%
Spring emerging nests	33	22.6%	50	29.4%
Overwintering nests that did not emerge	6	13.6%	4	2.4%
Unknown nests	11	7.5%	6	3.5%
Both fall and spring emerging nests	1	0.7%	0	0%



Figure 5. 2005 Lipid levels of hatchlings from the PIERP comparing fall emerging and spring emerging individuals. There was a lay date effect on energy reserves $(F_{1,28} = 7.65, P < 0.01)$



Figure 6. Differences in survivorship between fall emerging and spring emerging nest from 2004 -2007 on the PIERP. ANOVA test shows there was no season effect ($F_{1,406}$ =2.75, p >0.05). There was no significant year effect ($F_{3,406}$ =8.63, p <0.05), and no year by season interaction ($F_{3,406}$ = 1.7, p>0.05).



Figure 7. Lay date of fall and spring emerging nests for PIERP 2005-2007 nests. There was no lay date difference between fall and spring emerging hatchlings in 2005 and 2007 however there was a lay date effect on emergence time in 2006. A Wilcoxon rank sum test with continuity correction was used for 2005 and 2007 (2005: W = 1464.5, p-value > 0.5; N=128 fall emerged nests, N=23 spring emerged nests, 2007: W = 2933, p-value > 0.5; N= 108 fall emerged nests, N=50 spring emerged nests). A Wilcoxon rank sum test with continuity correction was used in 2006(W = 694, p-value < 0.05; N=62 fall emerged nests, N=30 spring emerged nests).

Discussion

Hundreds of cubic meters of sediment are dredged each year for commercial and recreational purposes which are then expelled into oceans, estuaries, rivers and lakes, or to land-based disposal facilities (Costa-Pierce and Weinstein, 2002). Opening new containment sites creates social and economic conflicts and presently, dredged material containment facilities are nearing capacity or are already full (Costa-Pierce and Weinstein, 2002). However, uncontaminated dredge materials are a valuable resource and can be used to create wildlife habitat islands and stabilize and restore beaches and wetlands (Costa-Pierce and Weinstein, 2002). Dredge material islands can be found throughout the Great Lakes, Pacific Coast, and in estuaries worldwide (Yozzo et al., 2004). Along the US Atlantic and Gulf Coast, over 2,000 dredge material islands can be found (Yozzo et al., 2004). Dredge material islands are used by shorebirds and wading birds as nesting areas and rookeries (Yozzo et al., 2004; Spear et al., 2007; Erwin and Beck, 2007; Piesschaert et al., 2005). Most of the research on dredge material island habitat has focused on population and community levels of avifauna (Yozzo et al., 2004; Spear et al., 2007; Erwin and Beck, 2007; Piesschaert et al., 2005). Few studies have focused on the use of dredge material habitat for reptiles, in particular chelonians. The PIERP terrapin study is the first to document the use of dredge material islands as creating suitable and possibly important habitat for turtles.

The Poplar Island Environmental Restoration Project (PIERP) is a unique opportunity to understand how large-scale ecological restoration projects affect terrapin populations and turtle populations in general. Since 2002 the long-term terrapin monitoring project has been conducted on Poplar Island to document terrapin nesting. By monitoring the terrapin populations on the PIERP, resource managers can understand how new habitat affects terrapin populations as well as understand how to create new terrapin nesting and juvenile habitat (Roosenburg et al., 2009). This information will contribute to understanding the ecological quality of the restored habitat on the PIERP, as well as understanding how terrapins respond to large-scale restoration projects (Roosenburg et al., 2009). The results of five years of terrapin nesting surveys reveals how diamondback terrapins use habitat created by the PIERP and how it has changed during that time.

This study surveyed potential nesting areas and followed nest fate throughout development to determine hatching success and hatchling quality. Terrapins began to use newly formed habitats for nesting after the perimeter dikes of Poplar Island were completed in 2002 (Roosenburg and Allman 2003; Roosenburg and Sullivan, 2006; Roosenburg et al., 2009; 2007; 2004). Nesting was restricted to areas where terrapins could access nesting sites. The stone dike around Poplar is a barrier that prevents terrapins from accessing many potential nesting sites (Roosenburg et al., 2004). Results show an increase in terrapin nests from 2002-2005 with a peak of nests in 2005. The number of nests per year now averages around 200 nests on Poplar, with the highest nesting density occurring opposite Coaches Island along Cell 5 and the notch. Terrapin nesting and juvenile habitat in the Poplar Island archipelago were reduced due to erosion (Roosenburg and Allman, 2003). Therefore, before Poplar Island Environmental Restoration Project (PIERP) began, terrapin populations in the area likely declined due to the emigration of adults and potentially reduced recruitment because of limited high quality nesting habitat (Roosenburg and Allman, 2003). Even alteration or damage to these habitats could negatively affect population dynamics (Roosenburg and Place, 1995). Results show that terrapins started using a suitable habitat as soon as it was formed. The newly restored wetlands could provide the resources that would allow terrapin populations to increase by providing high quality juvenile habitat (Roosenburg and Allman, 2003).

The proportion of nests surviving is consistent from year to year, with the highest survivorship occurring in Cell 6, then Cell 5, and lastly the notch. There is an increase in the number of nests in Cell 5 from 2005-2007 with a decrease in the number of nests occurring on the notch from 2005-2007. Suitable nesting habitat may become less available as more beach grass grows along the notch area. Increasing vegetation decreases terrapin nesting habitat in addition to making it more difficult to find nests.

Nest predation rates increased in 2005 and 2006 and then decreased in 2007. Fish crows began preying upon nests in 2005, in mid 2006 we began to protect nests by laying wire mesh over the nest and burying it less than 1cm. Protecting nests in this manner was adopted at the beginning of the nesting season in 2007 contributing to the high nest success during that year. Terrapins are preyed on by a whole variety of predators throughout their lifecycle. On Poplar Island nests are preyed upon by: beach grass (*Spartina* sp.), crows (*Corvus* sp.), corn snakes (*Elaphe gutta*), shrews, and ants (Roosenburg personal communication). Juveniles are preyed upon by shorebirds, wading birds and fish (Roosenburg personal communication). On Poplar raccoons are not

present. While bald eagles are present on the island, predation of adult terrapins has not been observed.

The percentage of nests that overwinter every year is about 30%, with almost the same emerging in the fall. Some nests are simply lost due to a number of reasons and their fate remains unknown. While one nest in 2006 exhibited both fall and spring emergence in one clutch, this is probably atypical. Only one hatchling from the nest emerged in the fall, while the rest of the clutch remained inside the natal nest to overwinter. Nests are only allowed to overwinter along cell 5 and the notch. Due to logistical factors all other nests are dug up at the end of fall to determine nest fate.

Hatchling lipid mass did not differ between fall and spring emerging hatchlings indicating there is neither an increased energy used or saved between the two overwintering strategies. Interestingly, lay date did affect lipid levels indicating that increased duration within the nest during the warm incubation period increases energy consumed by the hatchling, but regardless of lay date sufficient energy reserves remain for the hatchling to overwinter in the nest. Furthermore, these results indicate that lay date is not an important component in determining whether a nest overwinters or not. Gibbons and Nelson (1978) suggested that in species with facultative overwintering that earlier nests may be more likely to emerge in the fall and that nests laid later in the season would be more likely to overwinter. Our data does not support this hypothesis.

We also evaluated potential lay date using the multi-year data set from the notch and Cell 5. Again, overwintering does not appear to be determined by lay date (spring emerging hatchlings are laid throughout the entire nesting season). There is no effect of lay date on emergence time (fall or spring) in 2005 and 2007. However there was a lay date effect in 2006 where a greater proportion of nests laid early in the season emerged in the fall and those laid later emerged in the spring. Late season oviposition could result in insufficient number of days to complete embryonic development, and thus may affect emergence timing (Gibbons and Nelson, 1978). Our results indicate that date of oviposition's effects on emergence timing differ year to year. I therefore conclude that an internal clock set by date of oviposition does not stimulate nest emergence in *M. terrapin* hatchlings.

As results have shown, parts of Poplar Island are excellent terrapin nesting habitat, as indicated by the large number of nests, high nest survivorship, and high hatchling rate (Roosenburg et al., 2009). Poplar is unique because major nest predators such as raccoons and foxes are controlled, allowing for a much higher nest survivorship than normal. Also the lack of predators reduces the risk of predation for nesting females. The initial success of terrapin use on Poplar Island indicates that similar projects may create terrapin nesting habitat (Roosenburg et al., 2009). One of the major factors threatening terrapin populations throughout their range is the loss of nesting habitat to development and shoreline stabilization (Roosenburg, 1991; Siegel and Gibbons, 1995). Projects such as Poplar Island that combine the beneficial use of dredged material and ecological restoration have the potential to create habitat similar to what has been lost to erosion and human practices. With proper management, areas such as Poplar Island Environmental Restoration Project may become areas of concentration for terrapins and thus provide a source population for the terrapin recovery through out the Bay.

CHAPTER 2: TERRAPIN OVERWINTERING ECOLOGY

Winter is a time of physiological stress during which organisms employ a variety of survival strategies. Ectotherms most frequently try to overwinter in habitats where they can avoid freezing or they have unique adaptations that allow them to avoid the physiological stress of freezing. Most turtles avoid cold injury by retreating to habitats that do not freeze, and adult terrestrial turtles pass the winter underground in burrows (Utlsch, 2006). Aquatic turtles often burrow into the soft sediments of their aquatic habitat avoiding the freezing temperatures that occur near the surface (Utlsch, 2006). However, hatchlings of many aquatic species overwinter terrestrially (Draud et al., 2004; Packard and Packard, 2003), and when confronted by sub-zero temperatures, they use two methods to avoid injury from cold: supercooling and freeze tolerance (reviewed in Costanzo et al., 2008). In some species hatchlings emerge from the nest in late summer and early fall after completing embryonic development (e.g. snapping turtle *Chelydra serpintine*) while other species spend the winter as fully developed hatchlings in their natal nests and delay emergence until the spring (e.g. painted turtle *Chrysemys picta*; Gibbons and Nelson, 1978). Fall emerging hatchlings still overwinter terrestrially and must burrow into the sand to avoid cold injury (Draud et al., 2004; Draud, 2007).

Delayed emergence has been confirmed for five turtle families (Gibbons and Nelson, 1978; Costanzo et al., 1995; Ultsch, 2006). The benefits of late summer or fall emergence include the potential to immediately initiate feeding and growth (Gibbons and Nelson, 1978). The costs of immediate emergence include exposure to predators, inability to find suitable hibernating spots before the onset of cold weather, drying of aquatic habitats, and decreasing resources. On the other hand, delayed emergence and overwintering in the natal nest provides a period of growth and a sanctuary to avoid predation and emerge in an environment with increasing resources (Gibbons and Nelson, 1978; Ultsch, 2006).

Reasons for delayed emergence include the lack of rainfall and low temperatures (Gibbons and Nelson, 1978). Adverse ground conditions were observed to prevent emergence of C. picta in the fall and that rains are needed in the spring for ground softening (Hartweg, 1944; Gibbons and Nelson, 1978; DePari, 1996). Overwintering of clutches laid late in the nesting season may experience an insufficient number of warm days during the summer months in northern latitudes to emerge in the fall and hatchlings remain in the nest until the following spring (Gibbons and Nelson, 1978). Some species potentially delay emergence to avoid high environmental variability and uncertainty that exists for hatchlings that emerge in the fall (Gibbons and Nelson, 1978). Natural selection could favor individuals who use environmental cues (such as temperature or rainfall) to emerge facultatively during favorable conditions. Environmental cues (temperature or rainfall) were used by *Graptemys geographica* hatchlings to emerge into an environment with increasing natural resources (Nagle et al., 2004). Hatchlings may emerge in the fall if conditions for successful overwintering are lacking, suggesting that physiological mechanisms of cold tolerance and neonatal energy reserves are potential factors affecting delayed emergence (Nagle et al., 2004). Fall emergence maybe a response to poor structural or physical conditions that provide poor overwintering hibernacula (Nagle et al., 2004). The objective of this study is to compare environmental

parameters of fall and spring emerging nests of the diamondback terrapin (*Malaclemys terrapin*). Terrapin hatchlings delay emergence facultatively and thus they are an excellent model system to study potential causal mechanisms for emergence in hatchling turtles. Understanding this early life cycle stage for terrapins may help develop accurate ecophysiological models (Gibbons et al., 2001) that can help understand population dynamics and species distributions (Costanzo et al., 1995).

Emergence Timing in Hatchlings

Turtles are long lived reptiles that are successful in a variety of environments where they are exposed to extreme conditions such as dehydration, heat, cold, and hypoxia (Costanzo et al., 2008). The extreme conditions hatchlings must endure in the winter such as dehydration and injury from cold, has especially intrigued field biologists (Wyneken et al., 2008). In temperate species of turtles, eggs hatch in late summer and autumn (Costanzo et al., 2008). While some hatchlings emerge from the natal nest to seek other hibernacula, some species remain inside the natal nest (Costanzo et al., 2008). Timing of nest emergence is "different among taxa, populations, and even siblings sharing the same nest" (Costanzo et al., 2008).

As a strategy, "delayed emergence" occurs in five families and is practiced by 19 species, including *Malaclemys terrapin* (Gibbons and Nelson, 1978). There are a number of factors in the literature which may influence hatchling emergence timing in chelonians. Biological factors include internal timing and evolutionary response. Physical cues include; rainfall, temperature, nest entrapment, suboptimal incubation, and suboptimal hibernacula. However, there is little consensus about which of these factors is of greatest importance in emergence timing (Costanzo et al., 2008). Studies have shown that rainfall can influence emergence timing in three ways: 1) nest emergence happens to coordinate with precipitation due to the increase in soil moisture (Nagle et al., 2004); 2) rainfall could stimulate emergence by softening the soil (Wyneken et al., 2008); and 3) precipitation could flush out carbon dioxide from the nest and increase oxygen needed for locomotor activity (Costanzo et al., 2008; Wyneken et al., 2008). Temperature gradients in the soil could be a cue to synchronize emergence; where warmer temperatures encourage emergence and colder temperatures may induce overwintering. Nest entrapment is another physical cue, or barrier, that influences nest emergence timing. Studies have shown that nest emergence does not occur until rains have softened the soil in the spring after hatchlings have been forced to overwinter from the previous autumn due to hardened ground conditions (DePari 1996, Hartweg, 1944; Tinkle et al., 1981; Costanzo et al., 2008). Suboptimal incubation due to the physical characteristics of the nesting soil can affect emergence timing. Hatchlings may be developmentally immature and unprepared to leave the nest in autumn and therefore overwinter in the nest until the following spring. Suboptimal overwintering conditions such as flooding or degradation of the nest chamber may cause emergence in hatchlings (Costanzo et al., 2008; Nagle et al., 2004). Terrapin hatchling overwintering and facultative emergence has been observed on Polar Island, an environmental restoration project located in the middle Chesapeake Bay, since 2002 (Roosenburg et al., 2003).

Physiology of Overwintering and Soil

Survival of ectothermic animals at subzero temperatures depends on physiological and biochemical characteristics known as "cold hardiness" (Willmer et al., 2005; Schmidt-Nielson, 1997). Ectotherms use two general strategies for dealing with potential freezing of contained water: freeze tolerance and freeze intolerance (Willmer et al., 2005). Freeze tolerance is the ability to recover from extensive ice formation within the body (Willmer et al., 2005). Freeze tolerance is when ice forms and is limited to cellular spaces (Wyneken et al., 2008). Therefore, animals that use the freeze tolerance strategy depend upon ice inoculation at high subzero temperatures and a relatively slow cooling rate to limit ice to extra cellular spaces (Wyneken et al., 2008). Freeze intolerance is the ability to avoid ice formation in temperatures as low as -40° C to -50° C (Willmer et al., 2005). One way to avoid ice formation is to cool a liquid below its freezing point without it solidifying, known as supercooling (Packard and Packard, 2003; Willmer et al., 2005). Another way to avoid ice formation is to use antifreeze compounds that lower the freezing point without affecting the melting point (Schmidt-Nielsen, 1997). Most polar fish use antifreeze compounds in their blood and tissue fluids, which prevent the growth of ice crystals (Schmidt-Nielsen, 1997). Fish and most derived vertebrates are freeze intolerant (Schmidt-Nielsen, 1997). Along with many invertebrates, some amphibians (Hyla versicolor) and reptiles (Chrysemys picta) survive and tolerate ice formation (Schmidt-Nielsen, 1997). Whether turtles survive overwinter conditions by supercooling or freezing is debated (Packard and Packard, 2003; Costanzo et al., 2000; Storey and Storey, 1992). More recently, it has been stated that both survival methods may promote

survival in *Chrysmes picta* hatchlings (Costanzo et al., 1995). Studies on microenvironmental conditions and the effects that substratum has on hatchling survivorship may add insights about overwintering in turtles (Costanzo et al., 1995).

Any contact with ice would be lethal for a supercooled animal (Packard and Packard, 2001; Costanzo et al., 1995). Therefore, death by freezing in supercooled animals depends on temperature, presence of nuclei for ice formation, and time. When ice forms in an animal that has been supercooled, the crystals grow rapidly and cause extensive damage, puncturing cell membranes and disrupting subcellular structures and causing death (Schmidt-Nielsen, 1997). Ice is formed when a nucleus promotes organization of water molecules into an ice crystal lattice (Zachariassen and Kristiansen, 2000). The initial freezing is termed ice nucleation (Zachariassen and Kristiansen, 2000).

Ice nuclei form two ways: homogenous nucleation and heterogeneous nucleation (Lee and Costanzo,1998). Homogenous nucleation is the spontaneous aggregation of water molecules. The chance of aggregation increases with decreasing temperatures and the duration of chilling (Lee and Costanzo, 1998). Heterogeneous nucleation is when some other body, other than water, is the template where an ice crystal can form (Lee and Costanzo, 1998). These ice nucleating agents provide a place where water molecules congregate to form a nucleus where an ice crystal can grow; such as bacteria, fungi, and mineral crystalloids (Lee and Costanzo, 1998). The likelihood of ice nucleating agents in hatchlings depends on body temperature and various attributes of surrounding soil (Costanzo et al., 1998). Nesting soils host many ice nucleating agents which include organic, bacteria and fungi, and inorganic, crystalloids (Costanzo et al., 2000). Soil moisture has a strong influence on inoculation risk, because it determines the abundance of crystals in the vicinity of the turtle (Baker et al., 2006). Soil texture is also an important variable for overwintering hatchlings. Some ectotherms avoid ice inoculation better if the frozen substrate contains clay or organic matter which can absorb water and reduce the formation of ice in the pore space of soils (Costanzo et al., 1998). Moisture content, texture, and porosity directly or indirectly influence the abundance and distribution of ice within the substratum matrix (Costanzo et al., 1998). The presence of potent ice nuclei in nesting soils may impact winter survival demographics and geographic distribution of *C. picta* (Costanzo et al., 2000).

Materials and Methods

Study Species: Diamondback Terrapin, Malaclemys terrapin

The diamondback terrapin, *Malaclemys terrapin*, is an emydid turtle found along the United States eastern seaboard. Seven subspecies are found from Cape Cod, Massachusetts to Corpus Christi, Texas (Ernst et al., 1994). The northern diamondback terrapin, *Malaclemys terrapin terrapin* is found from Cape Cod, Massachusetts to Cape Hatteras, North Carolina (Ernst et al., 1994). Diamondback terrapins evolved in coastal habitats and with the retreat of the last glaciations expanded their range northward and inland (Roosenburg, 1994).

Throughout their range, terrapins nest on a variety of habitats above the mean high water mark (Roosenburg 1994, Roosenburg et al., 2003). In Maryland, terrapins, nest on elevated sand dunes on the coastal bays, and on narrow isolated sandy beaches found on the edges of salt marshes in the Chesapeake Bay and its tributaries (Roosenburg and Place, 1995). Terrapins can be philopatric to certain nesting areas within and among years (Roosenburg and Dunham, 1997), however they also are opportunists and will use new suitable habitat when it is available (Roosenburg, 1991). Diamondback terrapins are iteroparous, nesting as many as three times during the nesting season in the Chesapeake Bay (Roosenburg, 1991). Terrapins dig small flask shaped chambers and deposit an average of 13 eggs (Roosenburg and Dunham, 1997). Terrapins also have temperature-dependent sex determination (Roosenburg and Place, 1995). Finally, terrapin hatchlings facultatively overwinter in the nest (Baker et al., 2006) and thus the nest site selected by the female potentially have tremendous impact on the hatchling phenotype and the environment into which it emerges.

Study Site: Poplar Island Environmental Restoration Project

The Poplar Island Environmental Restoration Project (PIERP) is a large scale ecological restoration of a 450 hectare island that formerly existed in the middle Chesapeake Bay. Located near Tilghman, Maryland, the perimeter dike was completed in late 2001 and in the 2002 nesting season diamondback terrapins began to nest in the newly created habitat (Roosenburg et al., 2009). The PIERP provides a unique opportunity to study terrapin nesting ecology because mammalian nest predators are absent and therefore nest survivorship is extremely high. This allows for large sample size comparisons of fall and spring emerging nests and understanding the environmental factors that potentially influence timing of emergence.

Soil Sampling

I conducted a study to determine if a turtle's digging would disturb and alter the bulk density (soil mass per unit volume) of the soil. (Compaction raises bulk density, the amount of soil per volume g/cm³, while loosening of the soil lowers bulk density.) After nesting season, I created, two transects along the notch and Cell 5 that were above mean high tide line creating 40 pseudo turtle nests. Nests were dug within 13-17 cm, the average nest depth of terrapins (14.98 cm \pm 2.08 Montevecchi and Burger, 1975). Two flags were placed 18 cm on either side of the "pseudo nest" to relocate nests. Before retuning in the fall to take soil cores and get bulk density values, I used a computer generated random sample, to pick 20 out of the 40 pseudo nest to sample late November. I returned to collect soil cores; one soil core in the pseudo nest cores and 20 ground cores. Ground cores were used to compare bulk density values against pseudo nest cores in order to determine if a turtle's excavation would alter the compaction of the soil.

In order to compare fall and spring emerging nests, I used a computer generated random sample, to select 20 nests that emerged in the fall and 30 nests that delayed emergence. On November 26 - 28, 2007, I took soil cores from these nesting locations using a soil corer. A 3.8 cm pipe was used to take a soil core (18 cm) from the actual nest cavity, marked by a flag (if already emerged), or metal flashing (if hatchlings had not emerged). For each nest, I collected 2 samples (core A & B). In sample analysis, the means of core A and B were used for bulk density, porosity, and organic matter content. For texture and ice nucleating agents only core A were analyzed due to time constraints.

Cores were 14 cm deep, the average depth of terrapin nests (Montevecchi and Burger, 1975, Roosenburg 1991). Labeled plastic bags stored the samples that were transported back to Ohio University for analysis. Once back at Ohio University samples were placed in brown paper bags and left to air dry before analyses were conducted.

Nest Soil Analysis

Texture

Texture was determined by hydrometer method using Stokes Law on the settling time on the percentage of sand, silt, and clay. Hydrometers are read at 40 seconds and then again in 2 hours.

Organic Matter and Bulk Density

Organic content was determined from the mass of residue remaining after incinerating samples for 550° C for 4 hours. The mean bulk density (mass per unit volume) particle density (density of solid particles only) and porosity (percentage of pore space) was measured from weight of oven dried soil samples and the known core volume. *Inorganic Ice Nucleating Agents*

Costanzo (et al., 1998) procedures and methods were followed for analyzing soil ice nucleating agents. In order to test the activity of inorganic contents on ice nucleating agents, the temperature of crystallization was recorded. A quantity of air dried soil (100 mm³) was placed in a 5 ml polypropylene microfuge tube and 12.5 μ l of water (from reverse osmosis ultrapurification system) was added (Costanzo et al., 1998). The contents were mixed and then centrifuged (180g, 3 mm (1500 rpm for 3 minutes)). A 36 gauge copper-constantan thermocouple was taped to the tubes exterior. The tubes were then

placed in dry 20 ml test tubes. Samples were chilled by submerging the test tubes in a refrigerated glycol bath. Once samples were equilibrated to 0° C, they were cooled until water within the samples crystallized. The T_c (temperature of crystallization) of each sample was read from the output of a datalogger to which the thermocouples were connected. All microfuge tubes and utensils were autoclaved to eliminate organic ice nuclei.

Inorganic and Organic Ice Nucleating Agents

Water extractable ice nuclei was measured by washing each soil sample (0.5 g of water per gram of soil) until 10 μ l has been reached (Costanzo et al., 1998). Samples were centrifuged (180g, 3 mm (1500 rpm for 3 minutes)) (Costanzo et al., 1998). The supernatant was put through disk filter (5 mm) to remove fine particles. A 10 μ l sample of washings was drawn into the center of a 20 μ l capillary tube. The tube's ends were sealed with clay. Following the same procedure as before, a 36 gauge copper constantan thermocouple was taped to the side and then inserted into a dry 20 ml test tube. The tube was submerged in an ethanol bath. After samples equilibrate at 0° C, they were cooled until they froze. The potency of ice nuclei was estimated compared to the mean temperature of crystallization of washings with sterilized deionized water (Costanzo et al., 1998).

Statistical Analysis

Significance of statistical analyses was accepted at P < 0.05. Data were processed using Microsoft Excel and Sigma Plot and statistical analyses were conducted using R and Statistical Analysis Systems (SAS).

Results

Effect of turtle nesting on bulk density

Results of 20 pseudo turtle nests versus unexcavated ground cores along a transect in the notch and cell 5 show there is no significant difference between pseudo dug turtle nests and unexcavated surrounding ground cores. After and unsuccessful log transform was performed to normalize data with unequal variances of bulk density (g/cm³) a Twosample Kolmogorov-Smirnov test was performed (D = 0.35, p-value > 0.5; N=20 "pseudo" cores, N=20 "ground" cores).

Texture

Results show that there was no significant difference in percentage of sand silt and clay between fall and spring emerging nests from the randomly selected study nests out of the 2007 Poplar Island Nests (Figures 9 and 10). Comparison of fall versus spring emerging nests in sand and silt was conducted using a Wilcoxon rank sum test with continuity correction (Sand: W = 289.5, p-value = >0.05, Silt: W = 365.5, p-value = >0.05). Comparison of fall versus spring emerging nests for clay was done using a twosample Kolmogorov-Smirnov test (D = 0.2627, p-value = >0.05). Sample size was the same for fall and spring emerging nests for sand, silt and clay (N=21 for fall emerged nests N=29 for spring emerged nests).

Organic Matter

A Wilcoxon rank sum test with continuity correction found no difference in the organic matter between fall and spring emerging nests (W = 240.5; p-value = >0.05, N= 16 for fall emerging nests, N= 26 for spring emerging nests) (Figure 11).

Bulk Density

There was a difference in the mean bulk density values between spring emerging nests and fall emerging nests (Figures 12 and 13). Nests that emerged in the fall had lower bulk density values (looser, lighter soil) compared to spring emerged nests that had higher bulk density values (heavier, more compacted soil). A Wilcoxon rank sum test with continuity correction was reveals a significance (W = 182.5, p-value <0.05; N=21 fall emerging nests, N=30 spring emerging nests). A Wilcoxon ran sum test was performed because data was not normally distributed and had equal variances. *Inorganic Ice Nucleating Agents*

Using a repeated measures ANOVA, a difference in the temperature of crystallization between fall emerging nests and spring emerging nests was detected (Repeated ANOVA measures, p-value <0.0001; Figure 14).

Organic Ice Nucleating Agents

There was a difference in organic ice nucleating agents present between fall and spring emerging nests using a Wilcoxon rank sum test with continuity correction (W = 123, p-value <0.05; N= 16 for spring emerged nests, N=10 fall emerged nests) (Figure 14).

Lay Date

There is no effect of lay date on emergence time (fall or spring) in 2005 and 2007. A Wilcoxon rank sum test with continuity correction was used for 2005 and 2007 (2005: W = 1464.5, p-value > 0.5; N=128 fall emerged nests, N= 23 spring emerged nests, 2007: W = 2933, p-value > 0.5; N= 108 fall emerged nests, N=50 spring emerged nests). However there was a lay date effect in 2006 where nests laid early in the season emerged in the fall compared to nests laid later in the season, which emerged in the spring Wilcoxon rank sum test with continuity correction (W = 694, p-value < 0.05; N=62 fall emerged nests, N=30 spring emerged nests) (Figure 15).

Correlation Analysis

We conducted a correlation analysis to identify relationships among potential causal factors relating to fall or spring emergence. Variables included: lay date, clutch size, number of hatchlings, mean clutch mass, mean egg mass, mean hatchling mass, survivorship (number of eggs/ number of hatchlings), emergence time (spring or fall), sand, silt, clay, organic matter, mean nest bulk density values, mean nest porosity values, mean nest inorganic ice nucleating agents, and mean nest inorganic and organic ice nucleating agents.

Results show there is a correlation between survivorship and mean bulk density values (R = 0.427, p < 0.05, N=25); organic and inorganic INA and number of hatchlings (R= -0.450, p < 0.0408, N=21); silt and number of hatchlings (R= 0.298, p < 0.05, N=47). There is a negative correlation between sand and bulk density (R = -0.440, p < 0.002, N=47). There are also obvious correlations including: clutch mass and clutch size (R = 0.904, p < 0.001, N=28); hatchling size and clutch size (R= 0.484, p < 0.009, N=28); hatchlings and clutch mass (R = 0.507, p < 0.006, N=28); hatchlings and survivorship (R=0.785, p<0.001, N=25); clay and sand (R= -0.79, p < 0.001, N=47).



Figure 8. 2007 Spring and Fall emerging nests along the notch and Cell 5. Fall and spring nests tend to be clumped together in areas.



Figure 9. Percent of sand in 2007 Fall and Spring emerging nests. (Outliers are represented by black dots). Wilcoxon rank sum test with continuity correction (Sand: W = 289.5, p-value = >0.05; N=21 for fall emerged nests N=29 for spring emerged nests).



Figure 10. Percent of silt and clay in 2007 Fall and Spring emerging nests. (Outliers are represented by black dots). Wilcoxon rank sum test with continuity correction (Silt: W = 365.5, p-value = >0.05); Two-sample Kolmogorov-Smirnov test (Clay: D = 0.2627, p-value = >0.05). Sample size was the same for fall and spring emerging nests for silt and clay (N=21 for fall emerged nests N=29 for spring emerged nests).



Figure 11. Percent of organic matter in 2007 fall and spring emerging nests. (Black dots represent outliers). A Wilcoxon rank sum test with continuity correction (W = 240.5; p-value = >0.05, N= 16 for fall emerging nests, N= 26 for spring emerging nests).



Figure 12. Bulk Density of 2007 fall and spring emerging nests. (Black dots represent outliers). A Wilcoxon rank sum test with continuity correction (W = 182.5, p-value <0.05; N=21 fall emerging nests, N=30 spring emerging nests).



Figure 13. 2007 Fall and spring bulk density with a hot spot of emergence timing underneath from years 2004-2007. Fall emerging nests are light red and spring emerging nests are light blue. This is a visual representation showing areas with high bulk densities (more compacted) emerged in the spring compared to areas with low bulk density (less compacted) emerged in the fall.



Figure 14. 2007 Inorganic INA and Organic and Inorganic INA potency (as a measure of Temperature of Crystallization) in fall and spring emerging nests. Inorganic INA (Repeated ANOVA measures, p-value = <0.0001; N=9 for spring emerging nests, N=10 fall emerging nests). Inorganic and organic INA (Wilcoxon rank sum test with continuity correction W = 123, p-value <0.05; N= 16 for spring emerged nests, N=10 fall emerged nests).



Figure 15. Lay date of fall and spring emerging nests for PIERP 2005-2007 nests. There was no lay date difference between fall and spring emerging hatchlings in 2005 and 2007 however there was a lay date effect on emergence time in 2006. A Wilcoxon rank sum test with continuity correction was used for 2005 and 2007 (2005: W = 1464.5, p-value > 0.5; N=128 fall emerged nests, N= 23 spring emerged nests, 2007: W = 2933, p-value > 0.5; N= 108 fall emerged nests, N=50 spring emerged nests). A Wilcoxon rank sum test with continuity correction (W = 694, p-value < 0.05; N=62 fall emerged nests, N=30 spring emerged nests).

Discussion

M. terrapin nest emergence timing is affected by bulk density values and the presence of ice nucleating agents (as a measure of crystallization temperature). There was no difference in survival, oviposition, clutch size, texture (sand, silt, clay) and organic content in fall and spring emerging nests. Whether terrapin hatchlings overwinter in the nest as a result of compaction due to being trapped or because they remain due to good winter hibernacula as a result of characteristic INA is unknown. Fall nests have lower bulk density values and are therefore less compacted. Further studies on *M. terrapin* hatchling overwintering strategies (freeze tolerance or freeze intolerance) could prove useful in 1) identifying overwintering strategy method in field conditions and 2) identifying compaction (bulk density) as either enhancing hibernacula or as a barrier to emergence.

Results show that there is no difference from within nest survivorship from 2004-2007 on Poplar Island between fall and spring emerging nests. Therefore, one can conclude that natural selection is not favoring one emergence strategy over another in *M*. *terrapin;* which rules out the biological cue of evolutionary response as a factor that influences emergence timing.

Results show that date of oviposition did not influence emergence timing in either spring or fall emerging hatchlings in *M. terrapin*. Therefore, the date of oviposition, and indirectly, how late a female lays a nest in a season does not affect emergence timing in *M. terrapin*. Clutches laid later in the season were thought to overwinter because of insufficient number of warm days to complete development; therefore, hatchlings remain in the nest until the following spring (Gibbons and Nelson, 1978). Results show that an internal clock due to date of oviposition does not stimulate nest emergence in *M. terrapin* hatchlings. Internal timing is a biological cue that influences emergence timing in hatchlings; it is an internal "clock" that starts when eggs are laid until emergence.

Terrapin hatchling nest emergence time is related to the presence of ice nucleating agents. Using temperature of crystallization as a potency measure of ice nucleating agents present, fall emerging nests had ice nucleating agents (INAs) present in both the inorganic INA test as well the inorganic and organic INA test. Fall emerging nests crystallized at a higher temperature compared to spring emerging nests. Based on the percent of organic matter present, there is no difference between fall and spring emerging nests. Therefore, the inorganic ice nucleating agents present are the ones affecting the temperature of crystallization, and are driving the INA potency (more INAs are present if temperature of crystallization occurs at a higher temperature) rather than organic INAs. The presence of inorganic INAs, mineral crystalloids such as quartz and silica, found in sand may affect emergence timing in nests. It is possible that the INAs present in fall emerging nests may provide an adverse overwintering environment in supercooled animals.

Compaction can affect emergence timing in three ways: 1) the ground may be too compacted for hatchlings to successfully excavate; 2) indirectly effect embryonic development effecting temperature, hydric, and gas exchanges; or 3) compaction of the nest chamber influences the proximity of INA to hatchlings and therefore determines the condition of winter hibernacula. One physical factor influencing nest emergence timing is nest entrapment. Results show that fall emerged nests had a much lower bulk density value, and were therefore less compacted compared to spring emerging nests. Therefore, it may be probable that hatchlings that overwinter in their natal nest cannot break the nest chamber and are trapped. *Chrysemys picta* hatchlings were unable to penetrate the roof of the nest chamber when it has been hardened by the sun during the incubation period due to compaction (DePari, 1996). *Chrysemys picta* hatchlings from field nests in sand were more likely to emerge in the fall than hatchlings from nests constructed in soil (DePari, 1996).

Heat is an important resource for embryonic development and nest emergence activities. Thus thermal insufficiency may account for failure of some clutches to emerge in autumn. Porous well-drained soils can accelerate evaporative water loss (EWL), and heavier soils such as clays and clay loams retain moisture and thus tend to heat and cool relatively slowly (Costanzo et al., 2008). Compaction and more variation in clay may create a hypoxic environment for developing embryos. After hatching from eggs, neonates have energy reserves in the form of yolk lipids and proteins. Nest substrate may have an effect on the quality of yolk, since it is determined by both the amount of nutrients maternally invested and the thermal and hydric conditions during egg incubation. Perhaps *M. terrapin* in the "lighter" soil of fall emerged nests may heat quickly, which may be ideal for embryonic development but would remain unsuitable for overwintering. On the other hand, spring emerging nests may have had a better overwintering environment.

Compaction may also influence overwintering hibernacula. Porous soils contain large voids that can fill with ice, exposing hatchlings (Costanzo et al., 2008). While there was no difference in sand, silt, and clay in fall and spring emerging nests, there was a significant difference of compaction, and as result porosity. Nests that emerged in the fall had a higher porosity (lighter and less compacted) compared to nests that emerged in the spring. Compacted nests may be better overwintering hibernacula because INA (which would be detrimental for a supercooled hatchling) is kept away due to the compacted nest and the clay which would absorb water. In less compacted soils, hatchlings could be infiltrated with INA. Movement of hatchlings as they emerge from the eggshell may displace substrate above the nest chamber, which falls to the bottom, displacing hatchlings toward the surface. Studies found this phenomenon occurs in sea turtle nests, and that the effort of emergence from the nest chamber must be shared by siblings (Trullas and Paladino, 2007). However, in a well formed sealed chamber, the integrity of this chamber may not be compromised by the movement of hatchlings as they emerge from their eggs (DePari, 1996). Thus, friable soils are prone to infiltrating the nest cavity, placing hatchlings at an increased risk of freezing through INA contamination and inoculative freezing (Costanzo et al., 2008).

Malaclemys terrapin hatchling overwintering strategy

Whether hatchlings supercool or freeze as a strategy of cold hardiness is debated in literature (Packard and Packard 2001, 2003, 2004; Costanzo et al., 2006; Costanzo et al., 2000). Laboratory studies have found that *M. terrapin* hatchlings can use either freezing or supercooling as a method to survive subzero environments (Baker et al., 2006). Terrapins exhibit an extraordinary ability to supercool and remain unfrozen down to -15° C (Baker et al., 2006). Supercooling is also promoted by small body size. *Malaclemys terrapin* hatchlings are also capable of recovering from freezing at -2.5° C for at least seven days (Baker et al., 2006). They speculate that nesting soils have large amounts of INAs that would trigger freezing and death in supercooled turtles; therefore turtles would likely use a freeze tolerant strategy to overwinter (Baker et al., 2006).

Based on this study's results of compaction (more compacted nests emerged in the fall, less compacted nests emerged in the spring) and INA potency (nests that had higher amounts of INA emerged in the fall, nests that had lower amounts of INA emerged in the spring), it suggests that *M. terrapin* would use supercooling as a cold hardiness strategy in the field. Soil compaction may prove to be an effective barrier keeping INA away from supercooled hatchlings. Hatchlings that emerged in the fall in nests had high amount of INAs (as a measure of INA potency due to temperature of crystallization) present. Therefore, hatchlings would be actively avoiding overwintering hibernacula with high levels of INA present if they use the supercooling method.

Physical factors affecting terrapin nest emergence timing include; nest entrapment, suboptimal hibernacula (either due to compaction or presence/ absence of INAs), and possible suboptimal incubation environment. Biological cues such as evolutionary responses and internal timing do not seem to have an effect on emergence timing of *M. terrapin* hatchlings. This study further supports the conclusion that regional and local variation in soil characteristics can impact nest emergence timing in hatchling turtles (Costanzo et al., 2008). Further studies on biological and environmental factors that affect emergence timing are important in understanding the developmental environment and success of turtle nests (Wyneken et al., 2008). Future research may ultimately show that the driving force for hibernation emergence is an interaction between extrinsic and intrinsic influences.

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