

COMPARISON OF VIABILITY RATES, HATCHLING SURVIVORSHIP, AND SEX
RATIOS OF LABORATORY- AND FIELD- INCUBATED NESTS OF THE ESTUARINE,
EMYDID TURTLE *MALACLEMYS TERRAPIN*

THESIS

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ABSTRACT

Malaclemys terrapin, a member of family Emydidae, is one of only a few turtle species worldwide that lives in estuaries, and the only such turtle that inhabits the coastal United States. Diamondback terrapins feed primarily on mollusks and crustaceans. Due to their active foraging and mobility, terrapins are crucial macro-consumers in the estuarine ecosystems. Gateway National Recreation Area (GNRA), a large estuarine park east of the mouth of the Hudson River, NY has a large terrapin population. Due to habitat loss elsewhere, parks such as GNRA, are vital to the preservation of *M. terrapin*. Although terrapin numbers are declining throughout their range, the Jamaica Bay Wildlife Refuge (a unit of GNRA) population is still impressively large. Therefore it is vital to document the current viability rates, hatchling survivorship, and sex ratios of the JBWR population. I found that 46.1% of protected nests laid in JBWR were predated by raccoons despite the use of nest protectors, and 2.9% of the undisturbed nests were flooded. The average clutch size for terrapin nests (including protected nests and nests found post-hatching) over both years was 11.8 eggs. Egg viability for field-incubated nests was 88.6%, and 86.7% of the non-predated eggs resulting in hatchlings that emerged from the nest successfully (= emergence success). The viability rate for laboratory-incubated nests was 89.7%. Viability rates varied with incubation temperature, as did post-hatchling mortality and hatchling sex ratios. Sex ratios of laboratory incubated turtles varied with incubation temperature. At 26,28,30,32 and 34°C the sex ratio of hatchlings was 100, 93.3, 11.1, 0 and 7.7% respectively.

INTRODUCTION

Malaclemys terrapin, a member of family Emydidae, is one of only a few turtle species worldwide that lives in estuaries, and the only such turtle that inhabits coastal United States (Conant and Collins, 1991). Diamondback terrapins range from Cape Cod, Massachusetts to Corpus Christi, Texas with seven recognized sub-species, although none are reproductively isolated. There is significant variation in coloring between individual turtles even within a population, however one striking characteristic of this species is the extreme dimorphism between the sexes, with the smallest adult females being twice as large as the largest adult males (Montevecchi and Burger, 1975). This dimorphism is exhibited in other members of this family.

Diamondback terrapins feed primarily on mollusks and crustaceans (Tucker et al., 1995). Terrapins are crucial macro-consumers in the estuarine ecosystem due to their active foraging and mobility (Hurd et al., 1979). There is also some indication that *M. terrapin* and other beach-nesting turtles (i.e., sea turtles) play a major part in ecosystem resource cycling. By laying large numbers of eggs onshore, many of which will not survive, turtles deposit growth-limiting nutrients into beach ecosystems. Bouchard and Bjorndal (2000) showed that only approximately 25% of organic matter and 30% of inorganic matter deposited in sea turtle nests returned to the ocean as hatchling turtles. The remaining nutrients not utilized by the hatchlings (23% of nest mass) were absorbed by plant roots (Bouchard and Bjorndal, 2000). This may also occur in terrapin nests. Feinberg (2000) estimated that of approximately 22,000 terrapin eggs deposited on a 520ha island in New York, only about 4% returned to the sea as hatchlings. Stegmann et al. (1998) demonstrated that at least one plant, *Ammophila breviligulata* (dune grass), can absorb nutrients from *M. terrapin* eggs.

Because of its ecological significance as a consumer and a source of nutrients, the terrapin qualifies as a keystone species whose population status can be an indication of an ecosystem's health.

Although little is known about the aquatic ecology of *M. terrapin*, its nesting behavior has been well documented. On Long Island and New Jersey, the nesting season begins in early June and extends to the end of July (Burger, 1977; Feinberg, 2000). Diamondback terrapins nest primarily on isolated sandy beaches and vegetated dunes. Females come ashore primarily along with high tides (Feinberg, 2000) to seek out appropriate nest sites. It has been reported that nesting activity increases with high tides and increased temperatures (Burger and Montevecchi, 1975; Auger and Giovannone, 1979; Zimmerman, 1992; Feinberg, 2000). A female may test as many as ten sites with preliminary digging before the final nest is constructed. The number of rejected sites increases after rain when the sand is wet (Burger, 1977). The nesting process can take as long as several hours, and results in a nest that is wider than it is deep (Burger and Montevecchi, 1975).

Terrapin reproduction is consistent with a maternal condition-dependant, nest- site selection theory, whereby females with larger eggs are more likely to select nest sites that will produce female hatchlings, whereas females with smaller eggs are more likely to select sites that produce male hatchlings (Roosenburg, 1996). Roosenburg and Kelly (1996) determined that terrapin egg mass at oviposition is the primary determinant of hatchling mass. Hatchling mass is important because variation in mass can result in up to a three-year difference in age of first reproduction in female terrapins (Roosenburg and Kelly, 1996). Male terrapins in Louisiana reach maturity at about 9 cm in plastron length (~3 yrs) whereas females reach maturity at around 13.2-17.6 cm plastron length (after 6 yrs) (Cagle, 1952). In

New Jersey, egg-laying females range from 13.2-18.4 cm in plastron length (Montevecchi and Burger, 1975). Age of first reproduction is approximately six years for males in New Jersey, whereas age of first reproduction for females in New Jersey ranges from six to eleven years (Seigel, 1984). Although age and size of first reproduction varies with location, females consistently take longer than males to reach sexual maturity, and mature at larger sizes than males. Presumably larger hatchlings reach reproductive size more quickly, and because females are larger at maturity than males, selective oviposition would be a useful adaptive characteristic for nesting females, because it would optimize the growth of their offspring. Roosenburg and Kelly (1996) found support for this hypothesis when studying a Maryland terrapin population; however, the generality of these findings are unknown because and need to be examined in other turtle populations.

Once a terrapin has found an appropriate nest site she begins to dig the nest with her front claws. After forming an indentation she turns and digs with her hind legs. The first part of the nest formed is the neck, which she makes by scooping out substrate with her webbed hind feet. Diamondback terrapins lay their eggs in a flask-shaped nest (Fig. 1). The neck of the nest varies in length and can be dug straight down or at an angle. After the neck is formed the female makes the nest chamber by extending her webbed hind-feet and scooping out the substrate, which she piles at the mouth of the neck (Montevecchi and Burger, 1975). The chamber can be located directly below the neck or anywhere in a 360-degree radius of the neck entrance. The female lays her eggs when she has finished digging. The eggs may extend partway up the neck of the nest, depending on the size of the clutch and nest chamber. After she has finished depositing the eggs the female covers the eggs with excavated substrate. When the eggs are thoroughly covered the female spreads the disturbed

soil, leaving the nest thoroughly camouflaged. She does this by sweeping her hind legs across the surface of the sand as she slowly turns left and right, evenly mixing the disturbed soil into the surrounding sand (pers. obs.).

In some locations, terrapins may lay as many as three clutches of eggs in a nesting season (Feinberg, 2000). Clutch size appears to vary by location, ranging from 4 to 22 eggs. Females in the more northern regions produce larger clutches than those in the more southern part of the turtle's range (Ernst et al., 1994). Montevicchi and Burger (1975) reported a mean clutch size of 9.7 eggs in southern New Jersey, whereas Seigel (1980) reported a mean clutch size of 6.7 in Florida. On Long Island, Feinberg (2000) found the average clutch size to be 10.9 eggs.

Since the mid-nineteenth century diamondback terrapins (*M. terrapin*) have been commercially exploited for their meat, which was used mostly in soup stock. In the early twentieth century, demand for terrapin flesh reached an all-time high as such delicacies as turtle soup became the harbinger of a good economy (Ernst et al., 1994). These unprecedented demands led to the extirpation of many terrapin populations, especially those near large cities (Ernst et al., 1994). The terrapin populations on Long Island and adjacent areas were especially hard hit by this trend, due to their proximity to New York City and the fact that they were considered to produce premium terrapin flesh (Coker, 1951). By the mid-1930s Long Island terrapins had become so rare that they were considered locally extirpated (Marganoff, 1970). With the onset of World War I and prohibition, the demand for terrapins dissipated as such extravagances were less popular during the Great Depression (Marganoff, 1970). Over the past five decades terrapins have made a slow recovery and now occur in coastal habitats along much of Long Island (Morreale, 1992).

Since the reduction in hunting pressure early in the twentieth century, *M. terrapin* populations have increased. Despite this trend, however, the diamondback terrapin is still a species in jeopardy. Over the past fifty years there has been a substantial loss of terrapin habitat due to a variety of reasons, including the development of beachfront and estuarine areas. More than 12,000 acres of pristine tidal marshes (ideal terrapin habitat), 48% of the original habitat, were destroyed by development of Jamaica Bay, NY alone by the 1960s (Tanacredi, 1995). Along with this development has come an increase in the use of estuaries for recreational purposes such as fishing, crabbing, and boating. This increased activity has resulted in a dramatic decrease in optimal terrapin nesting habitat, as well as an increased risk to the turtles themselves. Turtles now encounter human-made floating debris, crab pots (which can trap and drown as many as 30 turtles per pot), boats, cars, and predators, which are attracted by human activities (Wood, 1997; Wood and Herlands, 1997; Miyata, 1980).

A large terrapin population still exists in Gateway National Recreation Area (GNRA), a large estuarine park east of the mouth of the Hudson River in NY. Because of habitat loss elsewhere, parks such as GNRA, and in particular wildlife refuges such as Jamaica Bay Wildlife Refuge (JBWR), are vital to the preservation of *M. terrapin*. GNRA is comprised of four management units spread over parts of New York and New Jersey. JBWR, the largest unit, is located on the southwest corner of Long Island and runs across the southern boundaries of the boroughs of Queens and Brooklyn. It consists of one large island, Ruler's Bar Hassock (RBH, 520 ha), and several smaller islands. Four of these islands were man-made and previously consisted of only salt marshes (Tanacredi, 1995). RBH is the only one accessible by automobile. It contains a visitor's center, research facilities and two brackish

ponds. Diamondback terrapins are highly visible at JBWR during the summer, and are an important part of the National Park Service's public outreach efforts (Taft, per. comm.).

Although terrapin numbers are declining throughout their range (Wood and Herlands, 1997), the JBWR population is still impressively large; Feinberg (2000) reported more than 1800 nests laid each year there. It is unknown whether this population is stable or changing. However, due to its size and the fact that the turtles are protected from commercial and recreational collection, JBWR is an excellent study site. Another reason that JBWR is of particular interest is that previous studies of RBH in the 1980s reported that terrapin nests were essentially never predated, and there were none of the usual nest predators (i.e., raccoons and foxes) found elsewhere (O'Connell, 1980; Cook, 1989). However, Feinberg (2000) found in 1999 that raccoons predated approximately 92% of all nests on RBH. Little is known about how raccoon became introduced into the refuge, or the exact size of the current raccoon population within the refuge, but raccoons are now common on RBH and have been found on one other island of JBWR (Ner, unpub. data). Raccoons are also suspected of attacking twenty-three female turtles that had a rear limb torn off and were eviscerated on RBH (Feinberg, 2000), which is a typical sign of raccoon attack (Seigel, 1980). This dramatic increase in predation has an unknown effect on the terrapin population.

Turtles, unlike most small vertebrates, are especially vulnerable to changes in the age structure of their populations. Due to the delayed maturity of turtles, there is a predominance of juveniles and sub-adults in most turtle population (Gibbs and Amato, 2000). Delayed maturation in turtles is caused by the demands for time and energy it takes to develop a protective shell. The development of a shell is directly related to survival rates. As turtles become larger they experience higher survival rates due to reduced predation, as compared to

other reptiles (Congdon et al., 1994). Therefore when many adults are removed from a population, specifically large females with high fecundity, the impact on the population demographics can be severe. Due to delayed maturation it will be several years before those breeding animals can be replaced, and their reproductive output can be replaced.

Furthermore, turtles lay their eggs on land where the mortality of eggs and hatchlings are high. Studies of loggerhead turtles (*Caretta caretta*) indicate ratios of eggs and hatchlings to adults as 400:1 and in snapping turtles (*Chelydra serpentina*) this ratio is approximately 18:1 (Gibbs and Amato, 2000). The ratios of eggs to adults indicate that few turtles will reach reproductive age despite the relatively high fertility experienced by turtles (Spotila et al., 1996; Gibbs and Amato, 2000). This means that if even a small number of reproductively mature animals are removed from a turtle population on a regular basis, it can have drastic results on the structure and persistence of that population.

Although it is commonly accepted that most turtles experience high egg viability rates (eggs that are able to produce hatchlings under ideal conditions), few published studies have compared viability rates of field-incubated nests to those of nests incubated in laboratory conditions. Generally speaking the stability of any turtle population appears to be more strongly influenced by adult and juvenile demographics than fecundity and nest survival rates (Congdon et al., 1994; Gibbs and Amato, 2000). Obviously, a pervasive failure of hatchling recruitment due to low fecundity or high predation rates can endanger an entire population (Spotila et al., 1996; Gibbs and Amato, 2000).

The viability rates of different *in situ* nests of alligator snapping turtles (*Macrolemys temminckii*) range from 24% to 48% (reviewed by Ernst et al., 1994), similar rates in box turtles (*Terrapene carolina*) range from 82% to 90% (reviewed by Dodd, 2001), whereas

spotted turtles (*Clemmys guttata*) exhibit a 68% viability rate *in situ* (Ernst, 1970). Previous studies of *M. terrapin* populations in New Jersey and Long Island have shown relatively low field viability rates (69%, Burger, 1976; 57% Feinberg, 2000, respectively) as compared to these other turtles. Many factors can cause low viability: lack of fertilization, developmental problems, contaminants, environmental disruptions (e.g., flooding and erosion) and high levels of pollutants. Such phenomena have been observed in alligators and red-eared slider turtles, caused by organic pollutants (Guillette and Crain, 1996). This may also occur in terrapins in JBWR because every day more than 320 million gallons of treated sewage and other materials, such as boat fuel and road runoff, are dumped into Jamaica Bay (NYC DEP New York Harbor Water Quality Survey, 1997).

The impact of the current intense nest predation rates (92%) (Feinberg, 2000) on the terrapin population at JBWR is unknown. Because adult turtles are long-lived, the population may continue to appear to flourish for many years despite the removal of some reproducing females, their nests, and the subsequent hatchlings produced. It may take as many as ten to fifteen years before a population decline becomes evident, when the remaining adults stop reproducing and there is an absence of younger turtles to take their place. By this point the damage may be irrevocable because there are not enough animals near breeding age to sustain the population.

Another result of this intense nest predation may be skewed sex ratios. Like many other turtle species, *M. terrapin* has temperature-dependent sex determination (TSD). Terrapin eggs from Maryland incubated at constant 26°C produced 100% males and eggs incubated at constant 32°C produced 100% females (Roosenburg and Kelly, 1996). Feinberg (2000) found that raccoons on RBH were more likely to predate nests laid in open, sandy

areas. This implies that a higher percentage of surviving nests were in locations with more vegetation and less solar exposure, thus resulting in cooler incubation temperatures and a hatchling sex ratio biased towards males.

Roosenburg and Place (1994) found that nest site conditions were vital in determining the sex of hatchlings. Of 185 hatchlings produced from artificial nests placed in various microhabitats, 184 of the hatchlings were male. They concluded that even “sunny” nests in areas without a southern exposure were likely to result in male hatchlings. This indicates that any sex-ratio bias caused by selective predation of terrapin nests in “warmer” areas may be exacerbated by the narrow microhabitat requirements needed to produce female hatchlings. Also, nests that are in less than optimal habitats may be exposed to temperature extremes, potentially affecting the viability of terrapin nests.

Furthermore, hatchling sex-ratio biases may lead to adult sex-ratio bias. Previous studies of other terrapin populations have reported adult sex-ratio biases favoring either males or females, depending on location (Lovich and Gibbons, 1990). Many explanations have been given for such a bias, including differential age to maturity and differential mortality due to human activities (e.g., crabbing). However, little information is available on the impact of nest predation on sex ratios and viability rates (Lovich and Gibbons, 1990; Wood, 1997; Roosenburg et al., 1997). It is unknown how biases in hatchling sex-ratios may affect later adult sex-ratios, nor is there anything known about sex-specific survivorship in hatchling and juvenile terrapins.

Like many other turtle species, diamondback terrapin hatchlings are not known to be sexually dimorphic and they do not have sex chromosomes. Therefore, it is difficult to sex hatchling and juvenile turtles without killing them so that gonads can be examined directly.

This complicates studies designed to determine whether or not factors such as predation and nest temperatures affect sex ratios of hatchling turtles when it is inappropriate to sacrifice animals. Wibbels et al. (2000) found that laparoscopy was the most reliable way to sex hatchling turtles of other species without sacrificing them, and that other techniques are unreliable, time consuming, and expensive. However, identifying the sex of live terrapin hatchlings using laparoscopy is complicated because they are so small. Burke et al. (1994) demonstrated that it is possible to sex hatchling gopher tortoise (*Gopherus polphemus*) using measurements of external features and a multivariate discriminating function. Such a function may be useful in helping to determine whether or not intense nest predation on terrapins may result in hatchlings of mostly one sex. It would also be useful for comparing laboratory work on TSD to fieldwork, especially when it is inappropriate to sacrifice hatchlings.

My objectives for this research were to 1) document the current viability rate, early hatchling survivorship, emergence success, and sex ratios of terrapin nests incubated in the field at JBWR, 2) document the viability rates, early hatchling survivorship, and sex-ratios of nests incubated at various temperatures in the laboratory, 3) compare the viability rates, hatchling survivorship, and sex-ratios of field- and laboratory-incubated nests, 4) develop a non-invasive technique to sex hatchlings in the field without harming them, and 5) test one component of Roosenberg's hypothesis (1996); specifically the relationship of adult female body size to egg and clutch characteristics (e.g., egg size, clutch size, clutch mass).

MATERIALS AND METHODS

Description of study area

Gateway National Recreation Area is composed of 10,500 hectares of land and water. This study took place in the largest management unit of the park, Jamaica Bay Wildlife Refuge (JBWR). NYCDEP (1987) and Feinberg (2000) characterized the dominant plant communities of JBWR as salt marsh, reed marsh, beach, dune, mixed-grassland, shrub land, and woodlands. Feinberg (2000) determined that the highest concentration of nesting terrapins at JBWR was found on Rulers Bar Hassock (RBH). I chose one area of RBH (~ 50 ha) as a representative of the entire island's nesting habitats because in previous years it contained the highest nesting density and it included all the habitat types found on RBH. The study site extended along the gravel trail from the visitor's center to the ninth bench on the gravel trail and from the shore of west pond to the estuary.

Locating Nesting Females

I conducted daily surveys from 1 June to 31 July in 2000 and 2001 during a six-hour period around the high tide closest to the solar zenith to maximize the opportunity of finding nesting females. On days with two daytime high tides the observation time was generally extended to cover both tides. Volunteer groups ranging from three to ten individuals provided assistance in covering the study area.

Groups of two to three people observed terrapins from shore with 7 x 35 field binoculars, from one of four monitoring stations. Whenever possible, vegetation was used as natural cover to observe terrapins without alerting them to our presence. Once a terrapin was located on land a person from the closest monitoring station was assigned to follow it. Observers kept approximately 10 m away from nesting females in order not to disturb them.

Observers followed females until they either nested or abandoned the nesting attempt. In either case females were captured, marked, measured, and palpated for eggs. Whenever time permitted a team of observers was assigned to survey the trails and surrounding areas for females that had come ashore unnoticed.

Protected Nests

When nesting females were located they were allowed to complete nesting only to the point that the eggs were covered, so that the nest chamber could be located easily. After the female was captured each nest was marked with an identifying flag placed just to the side of the incompletely covered nest so as not to puncture any of the eggs. The plastron length of captured females was measured and then the females were uniquely marked using the shell-notching method of Cagle (1939). In 2001 each female also was injected with a unique passive integrated transponder (PIT) in the body cavity.

Nests left *in situ* were protected using 65 x 85 cm² pieces of quarter-inch hardware cloth, which were anchored into the ground using six to ten 42 cm metal stakes. The hardware cloth was placed so that the nest chamber was centered under the center of the cloth to make it more difficult for nest predators to disturb the nest. An identifying flag was placed on the corner of the mesh, and the hardware cloth was thoroughly covered with soil so that it would be unlikely to absorb solar energy. Nests were covered with soil to their original depth. Gloves were worn to decrease the likelihood that human scent would attract nest predators such as raccoons.

Temperature loggers set to record hourly were buried in eight nests that represented three different solar exposure patterns: full sun (no shade), partial sun (shaded for two to four hours), and minimal sun (no direct sunlight anytime during the day). The temperature

loggers were buried next to the nest chamber. The excluder device was placed over the nest and temperature logger. Temperature logger data were compared over the same 30-day period, from 8 June 2001 to 7 July 2001.

Generally terrapin nests were not fully excavated before hatchlings emerged unless the eggs were removed to be incubated in the laboratory. Otherwise, protected nests were incubated in conditions that were as natural as possible. The excluder devices remained in place for approximately sixty days, and then were removed to enable hatchlings to emerge. Nests were monitored daily after the removal of excluders, and once the hatchlings emerged, the nests were fully excavated to determine the clutch size and the number of hatched, non-viable, and destroyed eggs, as well as dead hatchlings.

Hatchlings from nests that contained temperature loggers were trapped using a hardware cloth dome placed over the nest, after the excluder device was removed (~50 days). The dome was partially covered to provide shade for the hatchlings. Once several hatchlings had emerged, the dome was removed and the nest was excavated. These hatchlings were taken to the laboratory and reared.

Excavating Nests

After at least three hatchlings had emerged from each marked nest, the nest was fully excavated. Data also were collected from nests that were not observed being laid. This was possible because I found emergence holes that were caused by the hatchlings as they left these unmarked nests. Emergence holes (the indent left in the neck mouth) are very distinctive and easily distinguished from predated nests (Fig. 2). The shells of eggs that were dragged out of the nest by emerging hatchlings were collected carefully before excavation of the nest. The neck then was traced down carefully into the nest chamber and the chamber

was excavated. Eggshells, non-viable and destroyed eggs, and dead hatchlings were counted (Fig. 3). In cases where the shell fragments may have indicated a larger clutch size, the most conservative, yet plausible estimate of egg number was recorded.

Laboratory Incubation and Rearing

During 2000, all eggs from eight nests were incubated in the laboratory. Two of these were chosen for removal because they were laid below the tide line. The other six were chosen because they were the first or last nests laid on a day that they could be conveniently taken directly to the laboratory. Eggs from each clutch were randomly divided between five incubation temperatures: 26, 28, 30, 32, and 34°C. The eggs were uniquely labeled and placed in vented, closed, plastic containers containing damp vermiculite. The eggs were left in their respective incubators undisturbed except to dampen the vermiculite as needed to replace evaporated water. When an egg showed signs of hatching it was placed in its own cup. Hatchlings remained in the incubators until their yolk sacs were reabsorbed into their bodies. After the yolk sac was reabsorbed hatchlings were weighed, and each hatchling then was given a unique notch mark (Fig. 4).

Hatchlings were placed in rearing tanks containing salt water made with Corallife aquarium salt (6-8% salinity). Rearing tanks were 14 x 8.5 cm black plastic tubs with bottom drains. Fluval 4 filters containing crushed coral and fine mesh floss were used. Hatchlings were primarily fed chopped silversides, brine shrimp, shore shrimp, commercial turtle pellets, and occasionally live fishes, crabs, chopped squid, chopped clams, and whole mussels.

Maternal Affects Statistics

Prior to being placed into the incubation chambers, eggs were weighed and marked with a soft lead pencil in order to identify the egg and resultant hatchling with the female from which it came. Hatchlings were weighed 72 hours after hatching, and then again after two years. I used ANOVA to determine whether there was a significant difference in egg mass between females. Another ANOVA was performed in order to determine if larger eggs resulted from females with larger plastron size, and then another to determine if larger eggs produced larger hatchlings. Subsequent analysis using ANCOVA was performed in order to determine which had a greater affect on hatchling mass, the female which produced the egg, or the temperature at which the eggs was incubated.

Determining Sex Ratios

When hatchlings were approximately 20 g with a plastron length of 35 mm or above, their sex was identified via laparoscopic surgery. Hatchlings were first injected with 5 mg/kg of enrofloxacin (Baytril, Mobay Corp., Shawnee, Kansas) intramuscularly in the forelimb as a prophylactic against bacterial infection. Laparoscopy was carried out as described by Rostal et al. (1994). A 1.9mm O.D. x 10cm Lumina SL-telescope with 0° vision angle and a 150-watt light source (Karl Storz Endoscopy-America, Inc., Culver City, California USA) was used to identify gonads visually. Gonads were identified by morphology and coloration as described by Yntema (1981) and Roosenburg and Place (1994).

Morphological Sex Determination

I measured nine external morphological variables on each of 96 hatchlings, both alive and preserved, prior to laparoscopy. These were: anal width (AW), anal notch (AN), tail length (TL), post-cloacal tail length (PCTL), pre-cloacal tail length (PRTL), plastron width (PW),

plastron length (PL), carapace width (CW), carapace length (CL), and carapace height (CH) (Fig. 5). Each was recorded using digital calipers.

Anal width was measured as the distance between distal tips of the outside edges of the 12th and 13th scutes. Anal notch was the notch in the anal end of the plastron measured from the midline of the plastron to the outside edge of the notch. Tail length was measured from the base of the tail where it meets the body to the tip of the tail, and post-cloacal tail length was measured from the distal edge of the cloaca to the tip of the tail. Plastron length was measured along the midline of the plastron from its distal edge and plastron width was the measurement of the outer edges of the widest part of the plastron. Carapace width was the straight-line distance across the carapace from the 5th right scute to the 5th left scute, and CL was measured from the distal edge of the nuchal scute in a straight line to the notch between the 12th right and 12th left scutes. Carapace height was the distance between the plastron and carapace from the 5th scute.

These data were divided into two groups, those with a $PL < 30$ mm and those with $PL > 35$ mm because there were no individuals that fell between these groups. Following the procedure from Mertler and Vannetta (2002), stepwise logistic regression was used with sex as the dependent variable to develop equations that could be used to identify sex based on a combination of external measurements.

RESULTS

Field Data

During the course of this study one hundred and twenty-nine nests (2000 n= 68, 2001 n= 61) were protected *in situ*. Of those nests, 48.1% (2000: 40/68, 2001: 22/61) were predated by raccoons despite excluders, and 2.9% (2/69) of the undisturbed nests were flooded. Of the surviving 67 nests, 95% produced at least some hatchlings (= nest success). The mean clutch size for terrapin nests (including protected nests and nests found post-hatching) over both years combined was 11.8 eggs (median=11, mode=12, n= 67, SD=3.1). In 2000 the mean clutch size was 12.1 eggs (n=28, SD=3.2) and in 2001 the mean clutch size was 11.6 eggs (n=39, SD=3.1), there was no significant difference in clutch size between the two years ($t_{1,66}=0.5532$, $p=0.085$). A regression analysis showed that there was no significant relationship between clutch size and the date of egg deposition ($p=0.406$, $F_{1,31}=0.784$, $R^2=0.022$). Egg viability (the number of non-predated eggs that produced embryos) was 88.6% (639/721 eggs), and 86.5% (624/721 eggs) of the non-predated eggs resulted in hatchlings that emerged from the nest successfully (= emergence success) (Table 1). Regression analysis revealed no relationship between clutch size and emergence success ($p=0.320$, $F_{1,60}=1.006$, $R^2=0.016$). Hatchling mortality, the percentage of hatchlings that died hatching (hatchling sitting in opened egg with yolk sac) and hatchlings that died while emerging (hatchling out of egg with no yolk sac), combined was 2.1%.

In 2000 and 2001 combined, root predation resulted in the death of 34 out of 721 eggs that escaped raccoon predation, 4.7% of terrapin eggs (2000=5.6%, 2001=4.2%) incubated *in situ* (7 of 69 nests, 2000: 3/23 nests, 2001: 4/46 nests). There was no significant relationship between the probability of root predation and clutch size ($p=0.157$, $F_{1,60}=2.049$, $R^2=0.033$),

but all instances of root predation occurred in clutches of 11 or more eggs. Eggs were considered predated by roots if they were surrounded or penetrated by plant roots and did not produce a live hatchling. Roots of two plant species predated eggs: *Rosa rugosa* (salt spray rose) and *Ammophila breviligulata* (dune grass). *A. breviligulata* was involved in 64.7% of the root-predated eggs (2000: 100%, 2001: 36.8%). Its roots often surrounded the eggs and occasionally attached to the shell, but did not penetrate the eggs (Fig. 6). Five of fourteen eggs in one nest were completely surrounded by *A. breviligulata* roots, one egg on the outer edge of the surrounded group produced a viable hatchling whereas the other four eggs had well-developed embryos that did not survive to hatching (Fig. 7). *R. rugosa* predated no nests in 2000 and only one nest in 2001, and was therefore responsible for 35.3% of root-predated eggs (2000: 0%, 2001: 63.2%). Its roots penetrated the eggs and the eggs appeared to be desiccated. Eggs predated by *R. rugosa* were empty inside and only the eggshells remained, which were unusually chalky in texture (Fig. 8).

Laboratory Data

Among nine nests, a total of 107 eggs were collected for laboratory incubation in 2000, and 89.7% were viable (eggs that produce an embryo)(Table 2). Viability rates varied with incubation temperature but these differences were not significant ($X^2=0.48$, d.f.=4, $p>0.05$, Table 3). ANOVA indicated that incubation duration varied significantly with temperature ($F_{4,69}=37.81$, $p<0.0001$) (Table 3); the linear relationship between temperature and incubation duration was $y=-1.92+108.97$. The temperature with the highest viability was 28°C (100%) and viability decreased by approximately 5% for every two-degree (+/-) change in temperature (Table 3). The lowest viability was observed at 34°C (87%), this temperature also produced the smallest initial hatchling mass (mean=5.9 g, Tables 3 and 4).

Regression analysis confirmed that there was a positive correlation between initial hatchling mass and hatchling plastron length ($p < 0.001$, $F_{4,62} = 867.74$, $R^2 = 0.93$). ANOVA revealed that there was a significant difference in initial hatching mass between temperatures ($p < 0.001$, $F_{1,91} = 303.2$). An ANOVA also showed that this significant difference in hatchling mass between incubation temperatures persisted after two years ($p = 0.037$, $F_{4,59} = 2.86$; Table 4). Further analysis of the two-year old hatchlings using a Duncan's multiple comparison test revealed that the only group that differed in size were the hatchlings from the eggs incubated at 34°C (Table 5, Fig. 9).

Regression analyses showed no significant relationships between female plastron length and clutch characteristics such as clutch size, clutch mass, average egg size or hatchling mass (Table 6). However, egg size did vary significantly among female turtles ($F_{7,67} = 37.87$, $P < 0.0000$; Table 7). A subsequent ANOVA revealed that maternal effects had an influence on initial hatchling mass, specifically, that larger eggs produce larger hatchlings ($p < 0.0001$, $F_{38,36} = 8.34$) (Table 8). An ANCOVA revealed that maternal effects were more influential in determining initial hatchling mass than was incubation temperature, but that incubation temperature was strongly correlated with sex (Table 9, Fig. 10).

Hatchling Sex Ratios

Sex ratios of laboratory-incubated turtles varied with incubation temperature (Table 3, Fig. 11). At 32°C the sex ratio of hatchlings was 0% (0/13) male and at 26°C was 100% male (14/14). At 28°C 93.3% (14/15) of hatchlings were male and at 30°C 11.1% (2/18) of hatchlings were male. At 34°C 7.7% (1/13) of hatchlings were male.

I was not able to determine sex laporoscopically in 16.3% (8/49) of the turtles I examined because I was either unable to locate the gonads or because the gonads were

ambiguous in appearance. Animals whose sex could not be determined laporoscopically were excluded from any data for which sex was analyzed. There was a 2.1% (1/48) error in hatchlings I sexed laporoscopically and then examined by dissection. Hatchling size did not greatly affect the external appearance of the gonad in any way other than size (Fig. 12). The ovary was long, ribbon-shaped, grainy, and slightly reticulated in appearance. It appeared a translucent pinkish-yellow *in vivo* and a yellowish-white in freshly dissected specimens (Fig. 12). Testes were smooth, heart-shaped structures that were very pink in color both *in vivo* and in dissected specimens (Fig. 12). The survival rate for sexing hatchlings laporoscopically was 100%; all hatchlings were active and feeding forty-eight hours after the procedure.

Temperature Logger Analysis

Of the eight nests monitored with temperature loggers, only three escaped raccoon predation. Each surviving logger was from a different solar exposure pattern. The minimal solar exposure (MSE) nest (no direct sunlight) had an incubation duration of 63 days and an average daily temperature of 26.2°C, with an average minimum temperature of 22.3°C (daily range=16.7°C-25.9°C) and an average maximum temperature of 31.6°C (daily range=23.2°C-44.8°C). In the MSE 11/12 hatchlings were clearly males (sex of one hatchling could not be determined). Therefore sex ratio of this nest was either 91.7% male or 100% male. Incubation duration of the partial solar exposure (PSE) nest (shaded two to four hours/ day) was 69 days, average daily temperature was 26.4°C, average minimum temperature 21.4°C (daily range=16.7°C-25.9°C) and average maximum temperature of 31.8°C (daily range=23.2°C-42.4°C). In the PSE nest 4/15 hatchlings were clearly males, 7/15 hatchlings were clearly females, and 4/15 were of unidentifiable sex. If the four hatchlings whose sexes

were unidentifiable were males, then the sex ratio would be 53.3% male, if they were all female, the sex ratio would have been 26.7% male. The full sun (FS) nest (no shade) had an incubation duration of 61 days and an average daily temperature of 27.2°C, with an average minimum temperature of 22.2°C (daily range=16.3°C-25.9°C) and an average maximum temperature of 33.4°C (daily range=27.3°C-49.0°C). The FS nest resulted in a sex ratio of 0% male (0/3). The other eight eggs in the nest were destroyed by plant roots.

Morphological Sex Determination

Preliminary multiple linear regression was performed to calculate the Mahalanobis distance and to evaluate multicollinearity among the ten continuous predictor variables: anal width (AW), anal notch (AN), tail length (TL), post-cloacal tail length (PCTL), pre-cloacal tail length (PRTL), plastron width (PW), plastron length (PL), carapace width (CW), carapace length (CL), and carapace height (CH). The resulting table of regression coefficients indicated that the multicollinearity statistics exceeded 0.10 for all predictors except PL, CW, and TL; these three predictors therefore were removed from further analysis. The data were then divided into two groups: hatchlings with PL<30 (n=38) and hatchlings with PL>35 (n=59). Forward logistic regression then was conducted on the remaining variables (AW, AN, PCTL, PRTL, PW, CH, CL) to determine which independent variables were predictors of sex for each size group.

Hatchlings with PL<30

Data screening led to the elimination of two outlier cases (cases with extreme values of one variable that distort or unduly influence the model; Mertler and Vannatta, 2002). Regression results indicated two logistic models that were reliable statistically in distinguishing between the sexes ($X^2= 21.66$, $p=0.10$, removal=0.10). The first model used

four variables and correctly classified 80.6% (25 /31) of the cases whereas the second model used three predictors and correctly classified 77.4% (24/31) of the cases. Adding more variables to the equation did not increase its accuracy. Each model was translated into an equation where Y (sex) varies from one to two; Y values greater than 1.5 indicated males and values less than 1.5 indicated females. The models were:

$$\text{Model 1} \} \quad Y= 6.633 -0.75AW + 1.341PCTL + 2.023PRTL - 1.234CH$$

$$\text{Model 2} \} \quad Y= 6.276 + 1.155PCTL + 1.788PRTL - 1.444CH$$

Hatchlings with PL>35

Data screening led to the elimination of one outlier. Regression results indicated one logistic model that was reliable statistically in distinguishing between the sexes ($X^2=18.467$, $p=0.10$, removal=0.10). This model used one variable to indicate the sex correctly of 63.8% (37/58) of the cases. Adding additional variables to the equation did not increase its accuracy. The model was translated into an equation wherein Y varies from one to two; Y values greater than 1.5 indicated males and values less than 1.5 indicated females. The model was:

$$\text{Model 3} \} \quad Y= -3.408 + 0.205PCTL$$

DISCUSSION

Field Data

The egg-viability rate in field-incubated terrapin nests during this study (88.8%) was much higher than those found in previous field studies at the same location in New York (Feinberg, 2000) and in New Jersey (57% and 69%, respectively) (Burger, 1976). This may be an artifact of the sampling method used in these prior studies in which egg viability was determined using egg counts from unmonitored nests. As a result, egg count estimates may have been less accurate, because the emergence holes of these unmonitored nests may not have been found for several days, at which point, some of the eggshells from the nest may have been lost or degraded.

Average clutch size for terrapin nests at Jamaica Bay Wildlife Refuge (JBWR) in 2000 and 2001 were not significantly different from what Feinberg (2000) previously reported for this site, as was the percentage of nests lost to flooding. I protected nests from raccoons in the same manner as Feinberg (2000); I found egg viability (89.7%) for terrapin to be higher than those reported by Feinberg (2000) (57%). However, this is probably due to the small sample size of protected nests (five) in Feinberg's study (2000).

Surprisingly, I found no relationship between clutch size and date of oviposition. I expected such a relationship because terrapins laying two clutches per year had been documented at this site, and as many as three clutches per year was suspected (Feinberg, 2000). Therefore I expected that clutch sizes would decrease over the course of the nesting season. I hypothesized that females that laid multiple clutches would lay the majority of their fertilized eggs at the time of their first nesting when they would have ample time and resources to produce those eggs, and then lay the rest as they were ready. I theorized that females would lay the majority of their eggs in their first clutch due to the limited space

available for egg storage in the body cavity. This might leave a shorter period of time in which to prepare these second and third clutches for deposition and therefore they would not be able to lay as many eggs in subsequent nests.

Terrapin clutch sizes at this site varied from three to eighteen eggs, with the majority (68%) of clutches falling between nine and fifteen eggs. Emergence success has been positively correlated with clutch size in flatback turtles (*Natator depressus*) (Hewavisenthi and Paramenter, 2002). This is not the case with *M. terrapin*; there was no significant relationship between clutch size and emergence success. I suspect that this may be because *Natator* dig deeper nests ($\bar{x} = 58$ cm deep), compared to terrapins ($\bar{x} = 15$ cm deep; Montevecchi and Burger, 1975). *Natator* nests probably require more effort for hatchlings to escape; consequently it would be advantageous for *Natator* to have larger clutches that produce more hatchlings with which to share this effort. This would probably not be important in turtles that have shallower nests from which the hatchlings must escape.

Plant roots are a threat to both emerging and developing turtles because roots can halt development of embryos by absorbing moisture and solar heat before it enters the nest, thus preventing the nest from warming to normal incubation temperatures, and roots occasionally act as obstacles that trap emerging hatchlings. Plant roots also occasionally attach to and/or penetrate eggs and absorb nutrients from them, thus killing them (root predation). Two types of plant roots were found to predate terrapin nests at JBWR: *Ammophila breviligulata* and *Rosa rugosa*. Feinberg (2000) reported that 20% of eggs (7/35) were destroyed by roots in 2000. Of the 720 eggs laid in the nests protected in this 2001 study, plant roots predated 4.7%. This latter rate is more similar to that found for loggerhead sea turtles (*Caretta caretta*), which lost 5.3% of its eggs to sea oat grass (*Uniola paniculata*)

(Caldwell, 1959). I suspect plant predation rates vary considerably year to year, however, the high root predation rate reported by Feinberg (2000) for this study site may be an artifact of a small sample size.

Although Lazall and Aurger (1981) documented that *A. breviligulata* roots will occasionally penetrate the eggs they surround, it does not appear to grow actively towards and “digest” eggs. However, in the case of one nest in my study such growth was found; *A. breviligulata* roots completely surrounded all the eggs of the nest. When I excavated the nest, I discovered that only one egg in the center of the nest had been punctured, and it appeared that the roots grew around the nest and absorbed the nutrients from the destroyed egg while leaving the other eggs in the nest undisturbed. In another case it appeared that the roots of an *A. breviligulata* plant invaded a nest and halted the development of several of the eggs they surrounded as they grew. In this case five eggs in a nest of fifteen eggs were surrounded by dense root growth, one egg on the outermost edge of the surrounded group hatched, the other four eggs did not hatch and had late stage embryos inside them. Unlike *A. breviligulata*, *R. rugosa* appeared to actively “digest” the eggs its roots encountered, much like the *U. paniculata* roots described by Caldwell (1959). These root-predated eggs were unlike those predated by *A. breviligulata* in that they were crumpled in appearance and the eggshells were chalky in texture. Rootlets protruded from the eggs and it appeared that the exterior of the eggs were eroded away. When these eggs were dissected there was no yolk residue inside as had been found in the eggs that were predated by *A. breviligulata*.

The current intense nest predation (~92%) at JBWR (Feinberg, 2000) may not only threaten the terrapin population in the area, but also the stability of the beaches on which the terrapins nest. Terrapin eggs provide valuable nutrients to plants that are not normally

available in the nutrient poor, sandy soil of the refuge. Approximately 1893 terrapin nests are being eaten by raccoons on RBH every year, representing approximately 22,337 eggs (Feinberg, 2000). My data show that plants predate approximately 4.7% of oviposited eggs, thus raccoons are removing approximately 1050 eggs that would otherwise be predated by plants. Therefore a considerable quantity of eggs that would have provided additional nutrients directly to the plants is not doing so.

This may result in less plant growth, especially on the dunes and beaches where the majority of turtles nest. In the past several years, erosion has caused severe damage to the main terrapin nesting areas in the refuge (pers. obs.). A contributing factor for this may be a reduced growth of the plants that normally stabilize the dunes in the area such as *A. breviligulata* and *R. rugosa*.

Laboratory Data

Viability of terrapin eggs incubated in the laboratory averaged 89.7%, similar to my results in the field (88.6%). This was substantially higher than that reported by Feinberg (2000) (52%) for a laboratory-incubated nest from the same site. My results show that hatchling recruitment of terrapin nests at JBWR can be relatively high in the absence of predators, and may be able to sustain periodic heavy losses of nests to predators. It also suggests that the low viability rate found by Feinberg (2000) was an artifact of a small sample size or a poor incubation environment, and not indicative of a widespread failure in egg viability.

Viability rates varied with temperature, and were highest at the male-producing temperature of 28°C (100%). This temperature also had the highest post-hatching mortality (9.8%). The lowest viability rate was observed at the female-producing 34°C (87%), which

had the second highest post-hatching mortality observed (6.3%). Although these differences are not statistically significant, it appears that there is a negative correlation between egg viability and hatchling mortality associated with the embryonic development of turtles. Post-hatching mortality was highest at temperatures with the highest viability, perhaps because hatchlings that would not normally have been viable because of developmental problems were able to make it to hatching, but then succumbed to developmental abnormalities. In short, normal incubation temperatures simply delayed death of hatchlings.

Furthermore, perhaps mortality was high at the incubation temperatures with the lowest viability because hatchlings were incubated under environmental stresses (i.e., incubation temperatures outside normal range) during incubation that caused developmental abnormalities, which otherwise may have resulted in their mortality post-hatching. There was no post-hatching mortality experienced in the intermediate incubation temperatures because eggs with developmental anomalies did not develop to hatching, and the incubation environment caused no detrimental abnormalities. As a result, all normal (i.e., within the usual physiological range) incubation temperatures should result in approximately the same amount of hatchlings. This is supported by comparing the products of the viability and post-hatchling survivorship rates (i.e., total hatchling output) of hatchlings at all five incubation temperatures. The hatchling output ranged from 90.2-95.2% for temperatures within the normal incubation temperatures (26-32°C), and dropped to 78.1% for the physiologically stressing incubation temperature of 34°C. Further investigation must be carried out to conclude whether or not viability and post-hatching mortality is related to incubation temperatures. Specifically, a larger sample size is needed to determine whether or not this trend is real or artifactual.

Incubation temperature not only affected egg viability, but hatchling size as well. Cooler incubation temperatures tended to produce larger hatchlings. Although ANOVA revealed that this size difference did not persist after two years, an ANCOVA showed that there was heterogeneity in the slope of the hatchlings incubated at 34°C as compared to other incubation temperatures. This indicated that there was some difference in the growth rate of these turtles compared to the turtles incubated at the other incubation temperatures (Fig. 9). This is not unlikely because this constant incubation temperature is very high, and probably resulted in enough physiological stress to affect growth rate.

Surprisingly, there was no significant difference between the initial masses of male and female hatchlings within each treatment, except for those hatchlings incubated at 30°C. This is puzzling because cooler temperatures tended to result in larger hatchlings (males), and therefore it was expected that hatchling males would be larger. There are two reasons why this may not be the case. The first is that although cooler temperatures tended to result in males (pers. obs.), not all hatchlings incubated at these temperature were males, and vice versa. The other possible reason is that one temperature (34°C) strongly influenced the ANOVA results, because its hatchlings were much smaller than those produced at lower temperatures. Although cooler (male) temperatures tended to produce larger hatchlings, males incubated at 30°C were smaller than females at this temperature. This discrepancy may be due to the fact that 30°C is above the threshold temperature for producing female hatchlings. At this temperature female hatchlings may be better able to utilize their egg resources than male hatchlings, thus resulting in larger females at this temperature than males.

Previous studies (e.g., Roosenburg and Kelly, 1996; Brooks et al, 1991) revealed that maternal effects play a strong role in terrapin hatchling mass, with larger eggs producing larger hatchlings. As predicted by optimal egg-size theory (Smith and Fretwell, 1974), Roosenburg and Kelly (1996) observed that there was more variation in egg size between clutches than within. This is supported by my results; with maternal affects (egg mass) playing a more influential role than temperature in determining hatchling mass. As determined in previous studies (e.g., Montevecchi and Burger, 1975; Roosenburg and Dunham, 1997), egg mass was the primary influence on hatchling mass, however I found no relationship between female size and egg mass. As has been previously documented (Montevecchi and Burger, 1975; Roosenburg and Dunham, 1997), I found no relationship between female size and clutch size, female size and hatchling size, or clutch size and clutch mass.

Sex Ratios

Gonads from laboratory-incubated terrapin did not differ in appearance from descriptions in previous literature (Roosenburg and Place, 1994; Spotila et al., 1996). Of 96 hatchlings sexed, only one (incubated at 34°C) had ambiguous gonads. This temperature also produced one male hatchling, even though temperatures this high normally produce only female hatchlings (Roosenburg and Kelly, 1996). This unusual outcome along with the scute anomalies (e.g., missing or extra scutes) observed in many hatchlings incubated at this temperature, may have been a result of being incubated at an extreme temperature (Wood, pers. comm.). Unfortunately, I did not document these anomalies quantitatively. Similar scute abnormalities were observed in hatchlings that were incubated in my “hot” full-sun

field-incubated nest. Excluding these cases, the sex ratios of turtles incubated in the laboratory were similar to those previously reported (Roosenburg and Kelly, 1994).

Temperature-Logger Analysis

There has been some debate as to how temperature-logger data from field-incubated nests should be interpreted for study of temperature sex determination. Most researchers currently studying TSD in turtles have concluded that the relationship between temperatures in field-incubated nests and offspring sex ratios is complex, and no method has been found to describe this relationship mathematically that applies to multiple situations (Wilhoft et al., 1983; Bull, 1985; Schwarzkopf and Brooks, 1985; Mrosovsky and Provanha, 1989; Janzen, 1994a, 1994b; Souza and Vogt, 1994; Valenzuela, 2001). This is probably because temperature data sometimes cannot record the unique microclimates that are produced within natural nests accurately without affecting them, nor can they easily record the accumulation of metabolic heat that may influence sexual determination during the pivotal but narrow period in a turtle's embryonic development (Roosenburg and Place, 1994). This may have been demonstrated by my temperature-logger results. Roosenburg and Place's (1994) laboratory studies of Maryland terrapin nests determined that a constant average incubation temperature between 28.5°C and 29.5°C resulted in mixed hatchling sex ratios. Average temperatures of both my minimal solar exposure (MSE) nest and partial solar exposure (PSE) were below this temperature range, and these nests varied by no more than 0.3 degrees from each other in their daily measurements, yet the MSE nest resulted in the expected male hatchling bias and the PSE nest resulted in a mixed sex ratio. The third nest, the full-sun nest, resulted in the expected female-hatchling bias even though its average daily temperature was in the range expected to produce males. This result is suspect, however, because only

three of the eleven eggs in the nest were not destroyed by plant roots. Had these other eggs developed they may have resulted in a male-biased sex ratio as expected from the temperature-logger data.

Mrosovsky et al. (1999) suggested that a nest's incubation duration may be a fairly accurate indicator of sex ratios of field-incubated sea turtle nests because warm nests have a shorter incubation period and warm temperatures usually produce females. For example, Burger (1976) reported that incubation duration in terrapin nests was a function of nest temperature, with incubation duration ranging from 61-104 days depending on whether the nest was incubated in a warm or cool environment. However, this does not necessarily mean that incubation duration will be associated with sex ratios, because TSD is determined in a narrow period of time. My results do not support Mrosovsky et al.'s hypothesis. Their results may not be general across species or even between different nesting areas of the same species, because a nest with a shorter incubation duration may result in predominantly male hatchlings, if the nest's microclimate happened to be cool during the critical period and visa versa. Also nests with similar incubation durations may result in extremely different sex ratios, as in my MSN (predominantly male) and FSN (predominantly female) nests whose incubation duration differed by only two days (63 days and 61 days, respectively). This difference in sex ratio cannot be attributed to the time of egg deposition because the nests were laid within six days of each other in late June. It is also interesting to note that the PS nest was laid within the six-day period that the FS and MSN nests were laid, but it had the longest incubation duration of 69 days and resulted in a mixed sex ratio.

Morphological Sex Determination

Although laparoscopy was effective for sexing hatchlings, I found that it became difficult to find the gonads of the larger turtles. The procedure was time-consuming and required that hatchlings be kept for at least one day prior to the surgery and one day after. A typical surgery took between twenty minutes and an hour. Besides being labor-intensive and expensive, it is unknown whether or not this invasive technique may cause damage to the gonads that will hinder later reproduction. Other non-invasive sexing techniques such as testosterone challenge and DNA screening are more expensive and unreliable (Wibbels et al., 2000). The morphological approach to sexing hatchlings has many advantages over these methods. Once a reliable function has been developed it provides a non-invasive method to identify sex that is inexpensive, easy, and can be used under field conditions (Burke et al., 1994). Also, unlike some of the methods previously stated, it does not require the additional sacrificing of animals, if sufficient numbers of museum specimens are available to develop a function (Burke et al., 1994). This technique has been used to identify sex successfully in 95% of olive ridley sea turtles (*Lepidochelys olivacea*) and 78% to 99% of desert tortoises (*Gopherus agasizii*) (Boone and Holt, 2001; Michel-Morfin et al., 2001).

In this population of terrapin hatchlings, separate functions were developed for turtles of different size classes to increase the accuracy of the function. I developed two functions for hatchlings with plastron lengths <30 mm because, although the model 1 function was more reliable (80.6% accurate), it requires four measurements to identify sex, whereas the model 2 function is slightly less accurate (77.4%), but only requires three measurements. As hatchlings reached plastron lengths of >35 mm, another function was needed to sex them reliably. This function (model 3) is the least accurate (63.8%) of the functions developed but

requires only one measurement. This decrease in the power of the multivariate functions may be caused by morphological changes that disguise minor morphological differences that hatchlings undergo as they grow.

Multivariate functions are easy to develop and can identify otherwise inconspicuous dimorphisms in hatchling turtles. However, if there are important morphological differences between populations or within populations, new equations may be required for each sub-population to predict sex accurately. This may explain why the first two models developed did not predict sex accurately for larger turtles.

Another problem that may arise when using the morphological method to sex hatchlings from field-incubated nests may be subtle differences within a population of hatchlings caused by their nest site. For example, Solla et al. (2002) found that although there were morphological differences between the pre-cloacal tail length of male and female snapping turtle (*Chelydra serpentina*) hatchlings in sites contaminated by organochlorine pesticides (estrogen/androgen competitor), these differences were less pronounced than those found in hatchlings from non-contaminated sites and increased at a slower rate with body size than in non-contaminated sites. These subtle differences between hatchlings from different nest sites may decrease the strength of a multivariate function if only one function is developed for the entire hatchling population.

Historically, chelonians have been exploited for food, oil, traditional medicines, and other products. Increasingly during the twentieth century, the wildlife trade has been a contributing factor in the decline of many turtle species. Turtles are long-lived and remarkably hardy, and these facts have contributed to the public misunderstanding of the delicate life-history strategy typical of turtles. Often, the public's view of turtles is that of a

nomadic creature that can “carry its home around on its back.” This misconception often inhibits the ability to understand that turtles have specific habitat requirements and that their long-lives and iteroparity make them vulnerable to overexploitation (Frazer, 2000). The life-history strategy of turtles is adapted to occasional strong negative selective pressure on eggs and juveniles relatively low mortality among adult turtles. Unfortunately for turtles, humans have heavily exploited not only turtle eggs and juveniles, but adults as well. Human encroachment has also caused habitat destruction, fragmentation, and degradation, and has attracted subsidized predators, such as raccoons (Klemens, 2000).

Although diamondback terrapins are no longer threatened by overexploitation as a food source, they are now threatened by habitat destruction and degradation, as well as side effects of human encroachment such as subsidized predators, collection of animals for the pet trade, and deaths caused by human recreational activities (i.e., crabbing and boating). These new threats pose an even larger problem than overexploitation because they require a far more complicated approach to conserving this species than merely limiting the number of turtles that can be collected. It requires a systematic and multifaceted approach of identifying and protecting important feeding and breeding areas for this species as well as protecting the turtles themselves. To that end, this study will help determine which nesting beaches must be protected at JBWR, and how many nests are needed to sustain the current turtle population there. This study can also help in the formulation of a management plan for the refuge’s raccoon population.

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Table 1. Number of field-incubated nests. Number of eggs with visible embryos refers to the number of eggs that produce embryos, regardless of whether or not these embryos hatched. Number of emerged hatchlings refers to the number of hatchlings that successfully emerged from the nests. Number of non-viable eggs refers to the number of eggs that did not produce any noticeable embryo. Number of root-predated eggs refers to eggs destroyed by plant roots or completely enveloped by roots. The asterisk indicates that four of these eggs contained visible embryos. Number of hatchlings that died emerging refers to hatchlings that were found dead inside the nests. Overall fertility was 88.6%.

Number of nests	Total number of eggs	Total number of eggs with visible embryos	Number of emerged hatchlings	Number of non-viable eggs	Number of root predated eggs	Number of hatchlings that died emerging
63	721	639	624	52	34*	11

Table 2. Eggs sorted by clutch. Single asterisks indicate nests that were removed from below the high tide line. Double asterisks indicate eggs collected from partially depredated nests. These eggs were not included in any clutch size calculations.

Clutch ID	Clutch Size	Number Viable	Number Non-viable	Number Emerged	Number died post-emergence	Viability (%)
A	19	16	3	15	1	84.2
B	2**	2	0	2	0	100
C	15	14	1	14	1	93.3
D	16	16	0	16	0	100
E	13*	11	1	10	0	91.6
F	15	14	1	12	1	93.3
G	9*	7	1	6	0	87.5
H	12*	10	1	10	0	90.9
I	6**	6	0	6	0	100
Total	107	96	8	85	3	89.7

Table 3- Viability, mortality, and average hatchling mass at various temperatures. Viability refers to the number of eggs that contain visible embryos. Hatchling survivorship refers to the number of hatchlings that successfully emerged from their eggs. Post-hatching mortality refers to the number of hatchlings that died within 72 hours of emerging from their eggs. Total hatchling output is the product of viability rate and post-hatchling survivorship. Sex refers to the sex ratios of hatchlings at various temperatures in terms of percent males. Average hatchling mass refers to the mass of hatchlings 72 hours after hatching (SD). Numbers in parenthesis are sample sizes in all columns. The asterisk indicates that four eggs were lost due to an incubator malfunction.

Temperature (°C)	Viability (%)	Hatchling Survivorship (%)	Post-hatching Survivorship (%)	Total Hatchling Output (%)	Sex (%male)	Average Hatchling Mass (g) [+/-SD]	Incubation duration
26 (17)	94.1 (16)	100	100	94.1	100	6.9 [1.4] (16)	59.5 [2.9]
28 (22)	100 (22)	100	90.3 (20)	90.2	93.3	6.6 [0.9] (20)	57.4 [1.5]
30 (21)	95.2 (20)	95.5 (19)	100	95.2	11.1	6.9 [1.1] (19)	49.2 [0.7]
32 (20)	90 (18)	100	100	90	0	6.6 [1.0] (20)	45.3 [4.0]
34 (24)*	83.3(20)	80 (16)	93.7 (15)	78.1	7.7	5.9 [0.8] (15)	50.3 [4.0]

Table 4- Table of hatchling mass by temperature and sex at hatching and after two years. Asterisk indicates that table only includes weight data from individuals of known sex at two years.

Temperature (°C)	Avg. Hatchling mass (g)*	Avg. hatchling mass at 2yrs old (g)	Avg. mass of males at hatching (g)	Avg. mass of males at 2yrs (g)	Avg. mass of females at hatching (g)	Avg. mass of females at 2yrs. (g)
26	7.2 ±1.1	64.7±37.7	7.2±1.1	64.7±37.7	NA	NA
28	6.4±1.2	59.8±29.2	6.5±1.2	59.1±30.1	5.5±0	68.8±0
30	6.8±1.1	50.7±48.3	7.0±18.3	75.3±18.3	6.8±1.1	47.6±51.2
32	6.6±0.98	79.2±70.8	NA	NA	6.6±0.98	79±70.8
34	5.7±0.98	27.7±32.3	5.5±0	6.29±0	5.7±1.1	32±27.9

Table 5- Results of the Duncan's Multiple comparison post hoc test. Group refers to the temperature in degrees Celsius at which the hatchlings were incubated. Count indicates the number of individuals in the sample. Mean refers to the mean size of hatchlings after two years, and different from groups indicates which groups are significantly different from each other.

Group	Count	Mean	Different From Groups
34	6	27.77	28, 26, 32
32	11	76.345	34
30	18	50.721	
28	14	59.836	34
26	15	64.736	34

Table 6. Results of regression analysis of maternal characteristics. Clutch size sample was taken from both field- and laboratory-incubated nests and did not include disturbed or partially depredated nests. Clutch mass, average egg mass, and hatchling mass were taken from laboratory-incubated nests. Two nests were excluded from analysis because they were from partially depredated or incomplete nests.

Regression Analysis of Plastron size vs.	F	R ²	P	Significance
Clutch Size	(1,59) 0.047	0.01	0.5	none
Clutch Mass	(1,4) 1.305	0.246	0.3169	none
Average Egg Mass	(1,4) 0.5757	0.1258	0.4902	none
Average Hatchling Mass	(1,4) 0.1977	0.047	0.679	none

Table 7. Table of ANOVA results indicating that egg size varies significantly between females. Source indicates the source of the terms, DF indicates the degrees of freedom, and F indicates the F- ratio. Power of the test converges on 1.0.

Source	DF	Sum of Squares	Mean Square	F	Probability Level
Mother	7	100.5973	14.37105	34.87	0.00000
Eggs	67	27.61414	0.4121513		
Total	74	128.2115			

Table 8- Table of ANOVA results indicating that that there is a significant relationship between eggmass and initial hatchling mass. Source indicates the source of the terms, DF indicates the degrees of freedom, and F indicates the F- ratio. The power of the test converges on 1.0.

Source	DF	Sum of Squares	Mean Square	F	Probability Level
Eggmass	38	80.09237	2.107694	8.34	0.00000
Hatchmass	36	9.0975	0.2527083		
Total	74	89.18987			

Table 9- SAS output of Pearson correlation coefficients created by ANCOVA. Numbers on top indicate the correlation value, and the numbers below this indicate the probability level. The ANCOVA values are as follows: for temperature $F_{4,64}=12.11$, $P<0.0001$, for mothers $F_{7,64}=37.60$, $P<0.0001$, and for the interaction of mothers and temperatures $F_{17,64}=0.00$, $P=1.0$.

Pearson Correlation Coefficients, N = 64

	mother	hatchmass	sex	temp	eggmass
mother	1.00000	0.54947 <0.0001	-0.12723 0.3125	0.18677 0.1363	0.48997 <0.0001
hatchmass		1.00000	0.13227 0.2936	-0.26722 0.0314	0.89609 <0.0001
sex			1.00000	-0.76669 <0.0001	0.18941 0.1307
temp				1.00000	-0.21762 0.0816

Figure 1. a) Excavated terrapin nest. b) Diagram of a terrapin nest.

a



b

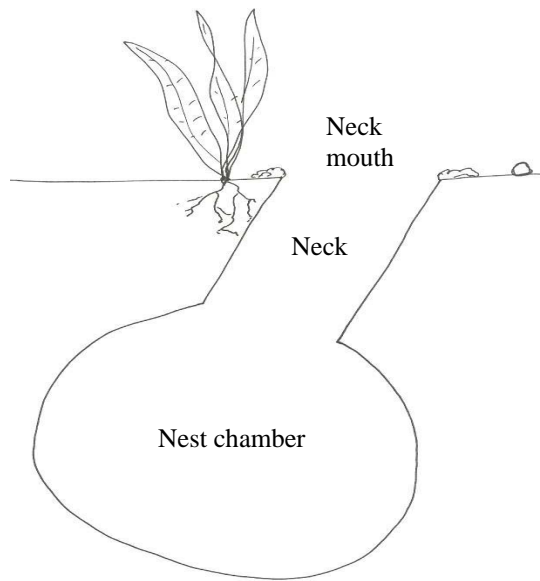


Figure 2- a) Emergence holes typically look like small indents in the ground with a trail of mostly intact eggshells leading away from the nest, b) Predated nests typically look like a large hole in the ground with eggshell pieces scattered around it. Often predated nests are accompanied by raccoon footprints and scat.

a.



b.



Figure 3- During excavation of a terrapin nest, all intact eggshells were lined up, and the eggshells of partially intact eggs were pieced together to determine the nest's clutch size.



Figure 4- Diagram of the notch marking system used to identify individual hatchlings.

Numbers on the inside indicate scutes, and numbers on the outside indicate values given to the scutes below them. A notch was made on the right side of the turtle to identify numbers in the “ones” section (one through ten) and subsequent marks were made on the left side of the turtle to indicate the “tens” and “hundreds” numbers (11 through 199). For example, number 133 would be denoted by placing notches in scutes 1, 2, 13, 23 and 24.

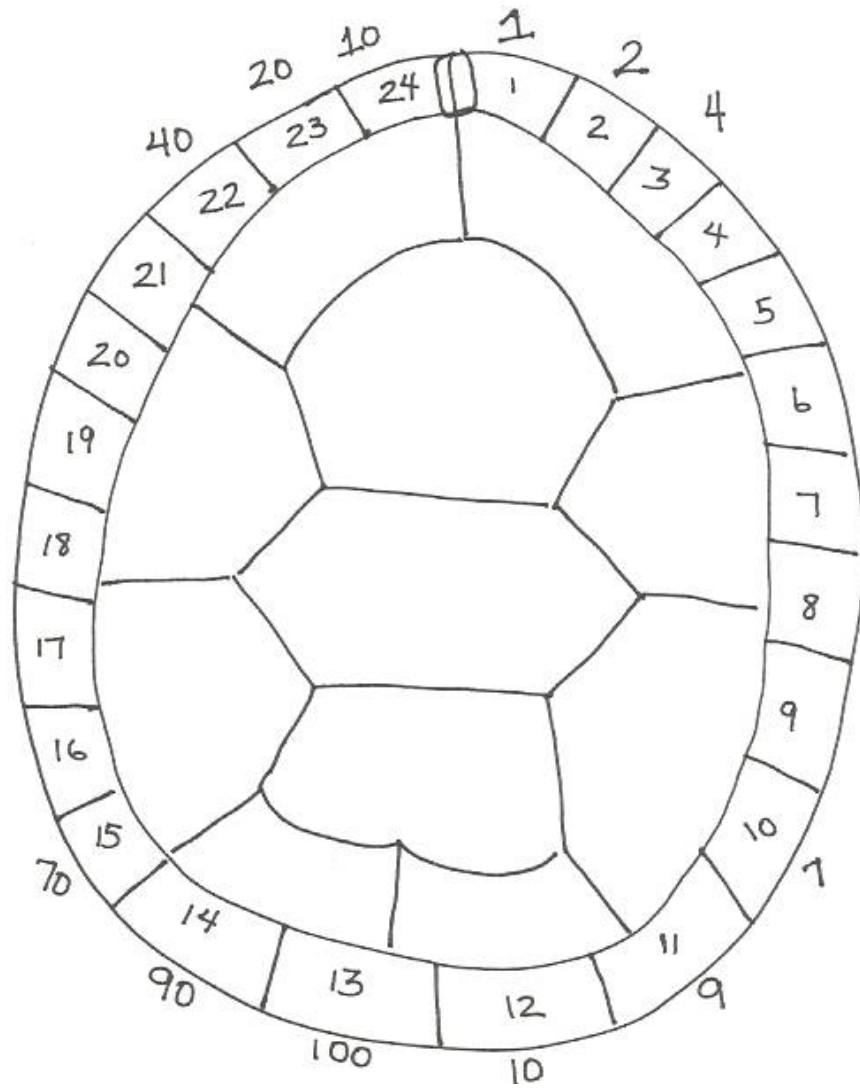


Figure 5a-c- Diagram of morphological measurements used to develop an equation to identify sex. Ten measurements were used: anal width (AW), anal notch (AN), carapace length (CL), carapace width (CW), carapace height (CH), plastron length (PL), plastron width (PW), tail length (TL), post-cloacal tail length (PCTL), and pre-cloacal tail length (PRTL).

Figure 5a.

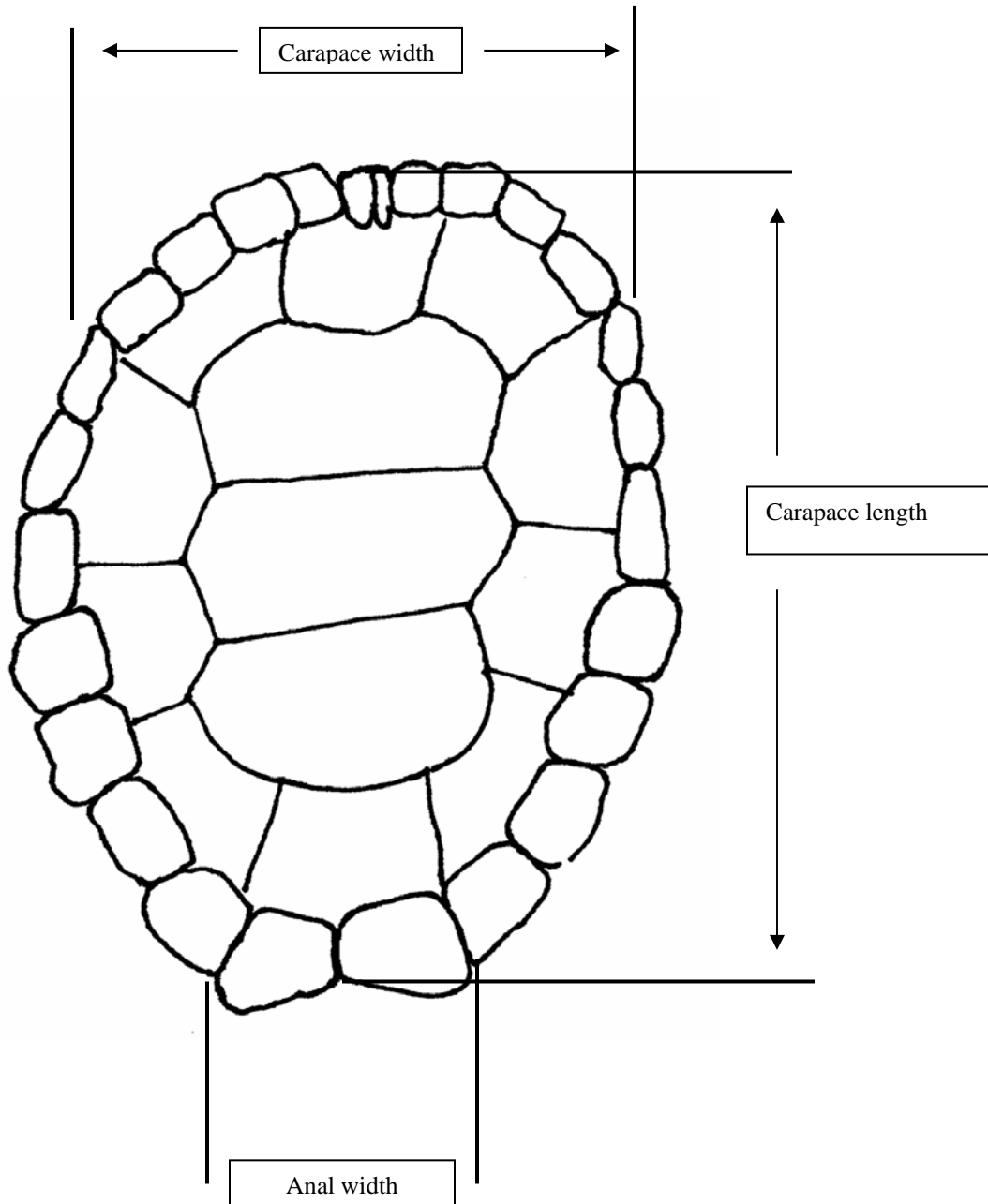


Figure 5b.

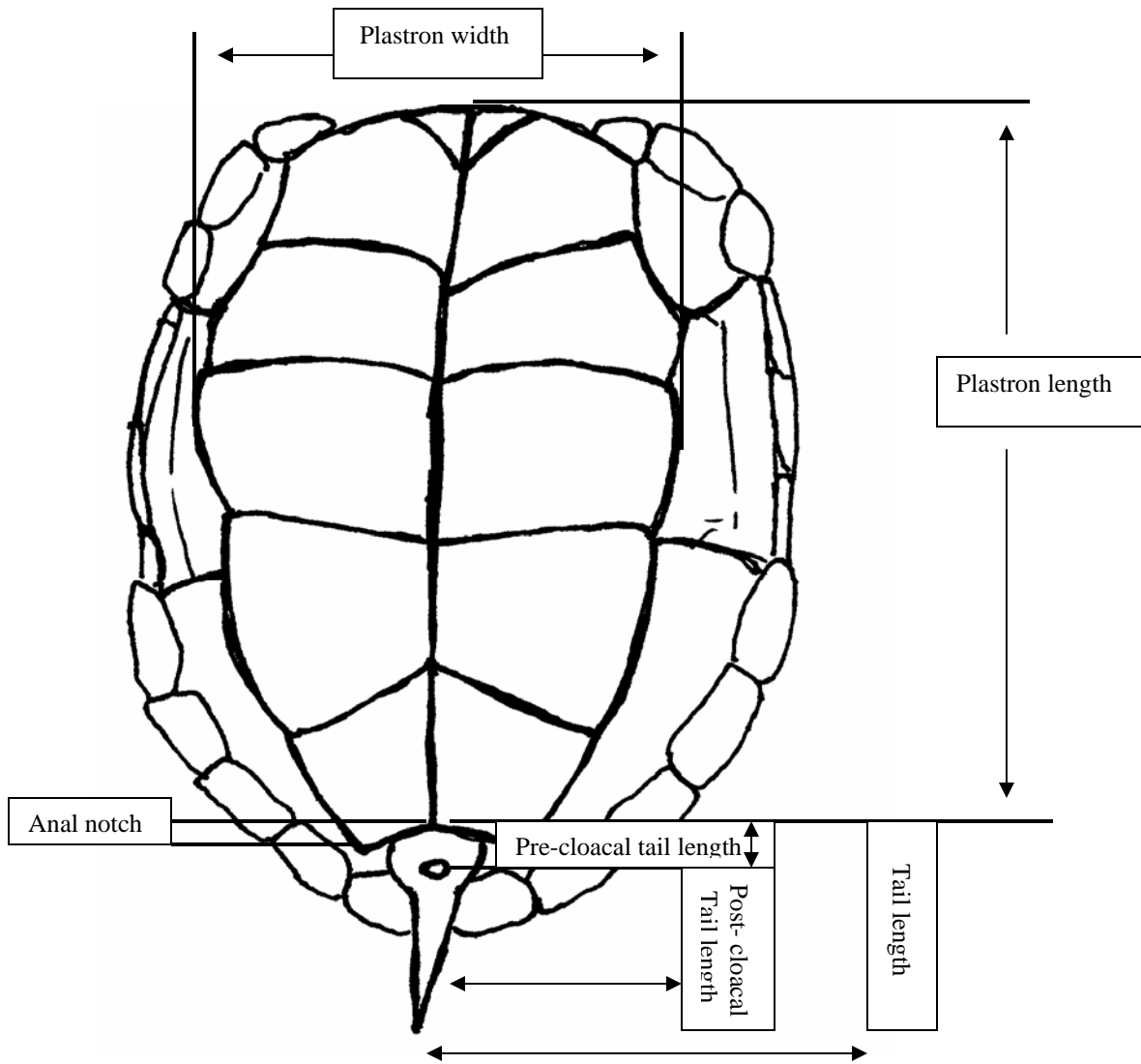


Figure 5c.

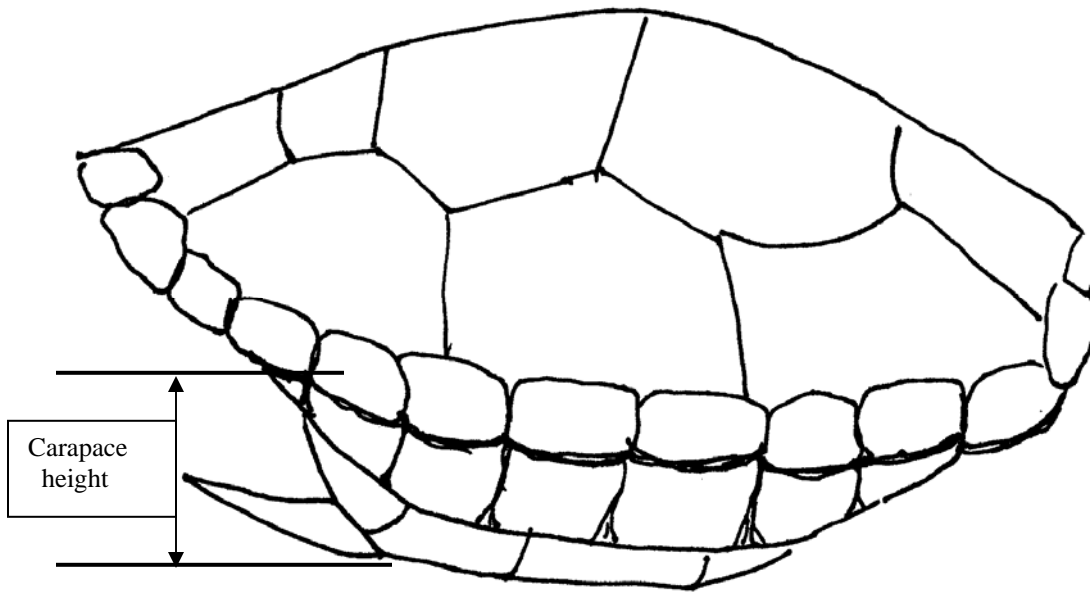


Figure 6- a) Terrapin nest that has been completely surrounded by *A. breviligulata* roots. b) Excavated eggs of the *A. breviligulata* nest, eggs are collapsed but otherwise intact, except for one (arrow) that retained its shape but was empty inside.

a



b



Figure 7. a) Partially root-predated nest. The highlighted area contains the four partially developed eggs that were destroyed by plant roots. b) Close-up picture of a partially developed egg from the above root predated nest. Arrow indicates the embryo of an almost fully developed hatchling.

a



b



Figure 8- Nest predated by *Rosa rugosa* roots. Arrow indicates one egg that was completely filled with plant rootlets.



Figure 9. Graph of average hatchling growth rate over two years at five incubation temperatures. X axis displays age in years, legend refers to incubation temperature in °C.

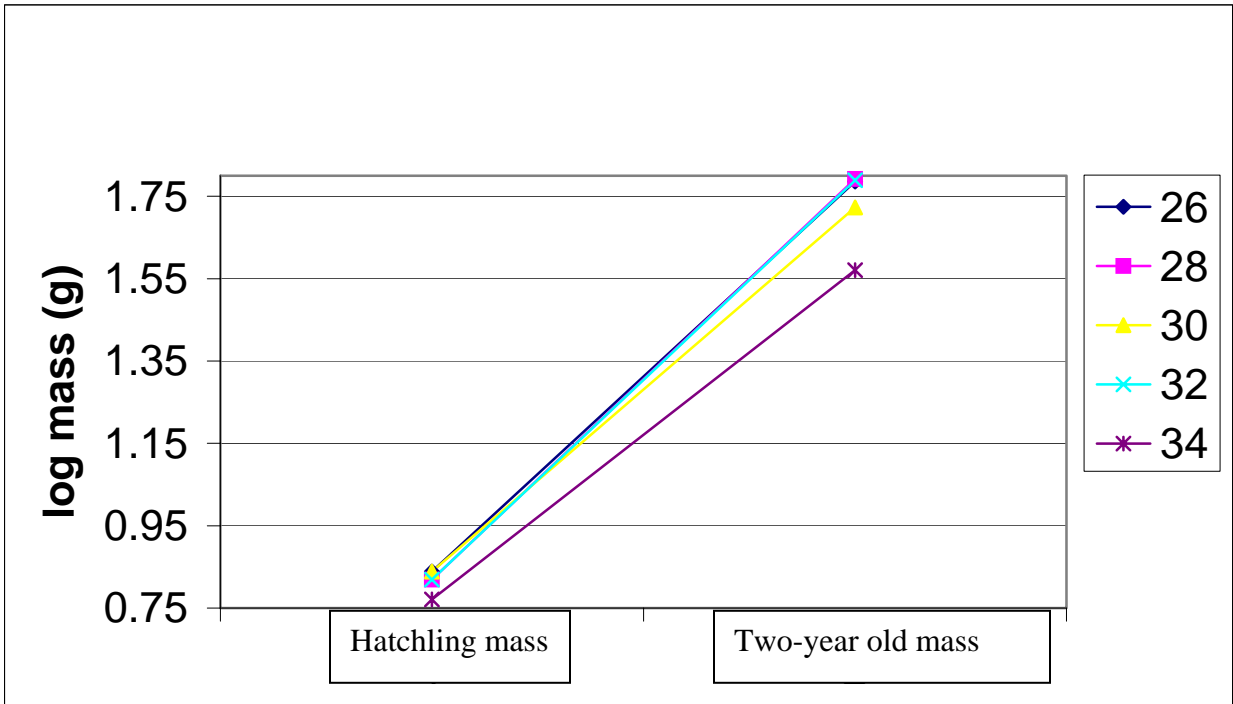


Figure 10. Hatchling size vs. egg mass categorized by mother. Each letter refers to a mother on Table 2. Eggs from each clutch were separated between five incubation temperatures 26, 28, 30, 32, and 34°C. Trend lines were placed into each clutch in the same color as its points. Eggs that did not hatch were not included in this graph.

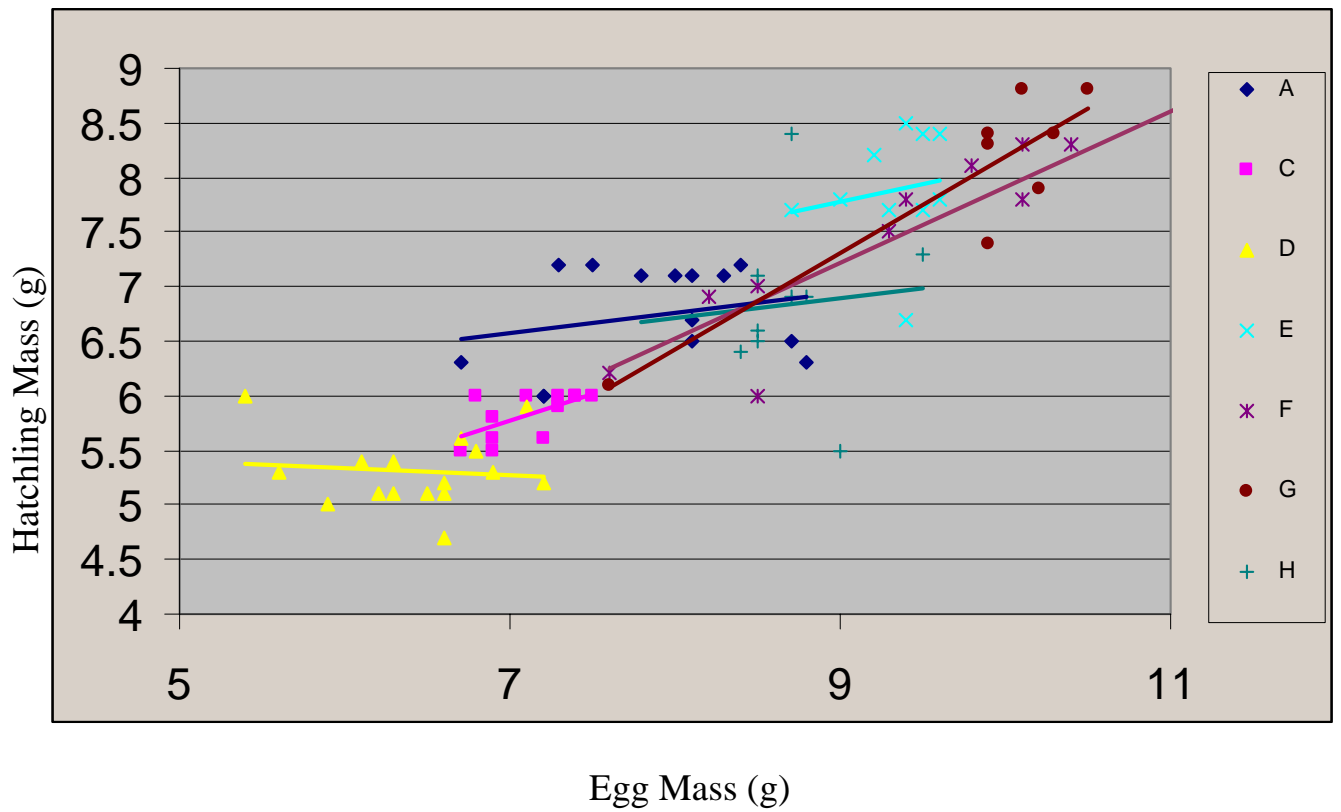


Figure 11. Graph of hatchling sex ratio in percent male. The dotted line represents 50% male.

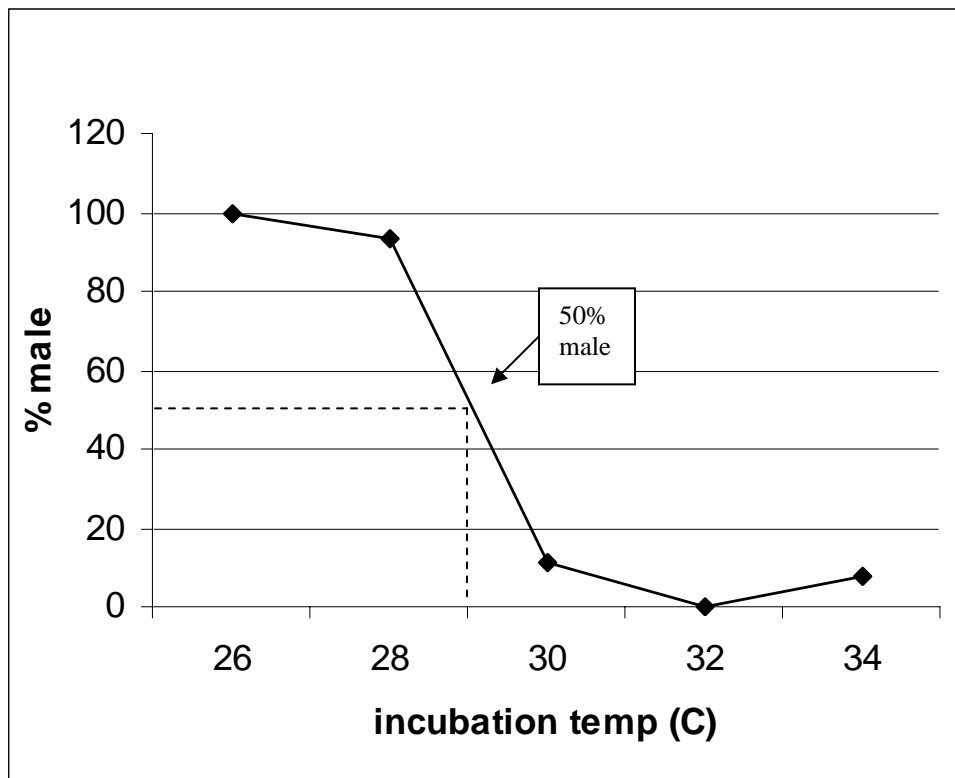
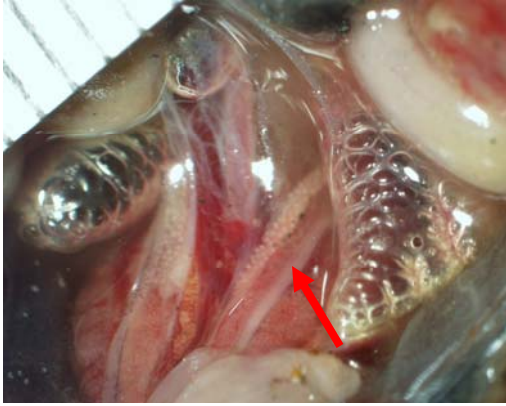
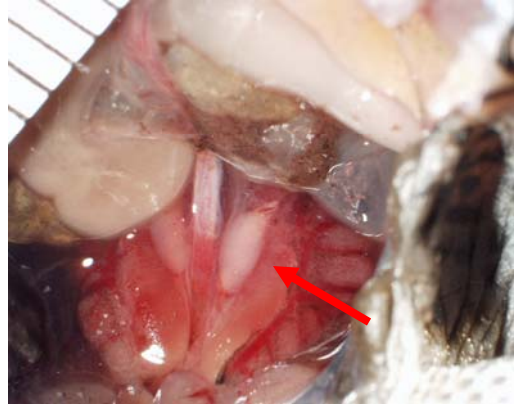


Figure 12. a) Immature ovary of a diamondback terrapin hatchling with a plastron length of 20 mm b) Immature testis of a diamondback terrapin hatchling with a plastron length of 21.5 mm c) Immature ovary of a diamondback terrapin hatchling with a plastron length of 38.5 mm d) Immature testis of a diamondback terrapin hatchling with a plastron length of 37.5 mm.

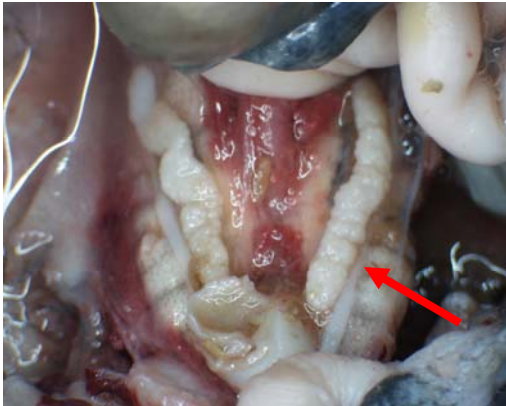
a



b



c



d

