# NESTING ECOLOGY OF THE

# TEXAS DIAMONDBACK TERRAPIN (Malaclemys terrapin littoralis)

By

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Dedication

To David and Cheryl George,

Parents that taught me to love and cherish nature



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# Abstract

#### NESTING ECOLOGY OF THE

#### TEXAS DIAMONDBACK TERRAPIN (Malaclemys terrapin littoralis)

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The Diamondback Terrapin is the only turtle in North America adapted to brackish water. The terrapin's range extends from Cape Cod, MA to Corpus Christi, TX and exhibit considerable latitudinal variation in life history attributes. Terrapin have small home ranges, but they can be difficult to locate, especially in Texas. Therefore little is known about the entire life history of terrapin. The objective of my study was to define what physical habitat attributes are associated with nesting terrapin, and when do terrapin potentially nest in Galveston Bay, TX. I used two lines of evidence including habitat surveys of known nesting areas and follicle development to accomplish these objectives. There is limited previous information on populations of terrapin in Galveston Bay, and terrapin have been observed nesting at each of our two study sites where we conducted nesting habitat surveys: Shell Island and South Deer Island. A Sokkia Total Station Set 330R and ArcGIS software was used to help collect and analyze geospatial data on multiple variables associated with predicted nesting habitat characteristics, including shell hash zone width (6-14 m), elevation, vegetation beyond shell hash, and sediment size and composition. Based on my assessment, two continuous areas were identified and delineated as possible nesting areas on Shell Island and seven possible nesting areas were delineated on South Deer Island. Each of these sites had high elevation (above 0.3255 m), high to medium shell hash zone width and high to medium levels of vegetation. Follicle size data were collected with a Sonosite® ultrasound from six different sites within Galveston Bay. Follicle development data were analyzed to identify seasonal nesting patterns. Based on follicle development trends, pitfall trap captures, and previous observations of terrapin nesting, nesting season was defined as starting from April to early June. Habitat attributes will be used in the future to define areas that most likely support nesting in the Gulf Coast.

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# Introduction

#### Background

The Diamondback Terrapin<sup>1</sup> (*Malaclemys terrapin*) is in the family Emyidae which contains seven sub-species distributed in estuaries from Cape Cod, MA to southern Texas (Glenn and Hauswaldt, 2005; Roosenburg, 1994). The Texas Diamondback Terrapin (*Malaclemys terrapin littoralis*) bears the sub-species epithet *littoralis*. Terrapins get their name for the diamond shaped scutes on their back. Researchers have attempted to use the concentric rings on the scutes to estimate the age of a terrapin. Unfortunately, when terrapin shed these scutes the concentric rings begin to smooth out, so older terrapin cannot be aged reliably using this method (Roosenburg, 1991). The maximum life span of terrapin is unknown but thought to be as long as 50 years, with little known about the first few years of the life (Roosenburg, 1991). It is the only species of turtle uniquely adapted to living in estuaries. They exhibit, however, latitudinal variation in microhabitat use within their range (Glenn and Hauswaldt, 2005).

Estuaries are located in between the ocean and upstream rivers. Typically, they are semi-enclosed, and have a continuous exchange of water with the open ocean (Roosenburg, 1994). An estuary is a unique habitat because of the mixing of freshwater inflow and marine water. This creates a gradient in salinity and suspended sediments, creating a dynamic physicochemical environment within the estuary (Pritchard, 1967). Variation in the amount of freshwater inflow and precipitation can alter the salinity

<sup>&</sup>lt;sup>1</sup> Henceforth interchangeably referred to as "terrapin"

gradient in an estuary (Pritchard, 1967). Astronomical and wind influenced tides can reinforce or partially neutralize the influence of the factors listed above (Pritchard, 1967). Terrapin are the only reptile found in estuaries that are known to have a functional salt gland, an exocrine gland that aides the kidney by producing excretions containing higher concentrations of salt than sea water (Davenport and Macedo, 1990). Terrapin need a balance of Na<sup>+</sup> and Cl<sup>-</sup> ions to prevent diffusion of unwanted or essential fluids in or out of the body, which is controlled by gradients that are regulated by the salt gland (Davenport and Macedo, 1990). Dunson (1970) confirmed the existence of the terrapin's salt gland by transferring terrapins from freshwater to 3.3 % NaCl solution and recording an increase in electrolyte concentration of whole blood. However, Davenport and Macedo (1990) states that the terrapin's salt gland is aided by behavioral osmotic control because the gland is not as effective as in true marine reptiles such as sea turtles. Terrapins will drink water from surface layers of freshwater overlying more dense saline water and from pooled rainwater (Davenport and Macedo, 1990). Dunson (1970) reported that terrapin have been collected from Maryland to Florida where found in salinities between 11 and 32 parts per thousand.

Terrapins appear to have limited dispersal ability and small home ranges (Roosenburg et al, 1999). However female terrapin move farther and spend more time in deeper water than male terrapin (Roosenburg et al, 1999). They appear to be able to adapt to the local microenvironments found in estuarine habitats (Seigel, 1984). In Texas, terrapin are found around saltmarshes dominated by *Spartina alterniflora*, and can be found burrowed along adjacent tidal creek edges (Clarkson, 2012; Haskett, 2011; Hogan, 2003; Koza, 2006). During the warmer months in Texas, terrapin can be found

swimming in tidal creeks and in open bays. They are thought to move into tidal creeks for mating. During colder months in Texas terrapin burrow into the mud and aestivate. Recently, some terrapin have been found in "social burrows" in which many terrapin are burrowed in the same hole (Clarkson, 2012; Pers. Obs.).

It has been suggested that terrapin's habitat selection is driven by prey availability (Roosenburg et al, 1999). Marsh periwinkle snails (*Littorina irrorata*) seem to be the main food source of terrapin, but they were observed eating fiddler crabs, small fish, clams, and other estuarine invertebrates (Roosenburg et al, 1999). Roosenburg et al (1999) discussed the differences in diet between populations of terrapin in the Patuxent area and South Carolina. The South Carolina population fed primarily on marsh periwinkle snails, the main food source of the terrapins. In contrast, the Patuxent population primary food source was soft shelled clams, razor clams and other small clam species. However, most of the published data suggest that terrapin feeding habits are similar throughout its range (Seigel, 1984). Dunson (1970) found that terrapin distribution is more influenced by salinity than prey composition or availability. However, the presence of a functioning salt gland may help reduce the influence of salinity on habitat selection.

The Diamondback terrapin is not federally listed as endangered or threatened (Mitro, 2003). However, distinct populations and sub-species have been provided protection by selected states (Mitro, 2003). The diamondback terrapin is considered vulnerable to local extinctions because of low nest survival, and delayed maturity, apparent specialized adaption to declining estuarine wetland environments, high nesting site fidelity, limited home range, and having temperature dependent sex determination

(Glenn and Hauswaldt, 2005). Based on a study by Roosenburg (1991), a female terrapin must reproduce at maximum output to replace herself as a hatchling in the population. In addition to local extinction risk due to biological factors, other threats include drowning in crab pots, habitat fragmentation and destruction, nesting female mortality associated with vehicle collisions, boat collisions, and possibly pollution (Bishop, 1983; Roosenburg et al, 1997). The terrapin's habitat and range overlaps with that of the blue crab (Roosenburg et al, 1997). Therefore, terrapin deaths from drowning in crab pots are frequent across its range, and there have been reports of up to 50 terrapin dead in one trap (Roosenburg et al, 1997).

In the past, local populations of terrapin have been reduced due to over harvesting (Bishop, 1983). Terrapin were regarded as a culinary delicacy and populations began declining in the 1800s (Hogan, 2003). Today the commercial harvest of terrapin is either banned or regulated in most states to insure the survival of the species (Bishop, 1983).

Butler et al (2004) and Mitro (2003) have reported nest predation from gulls, raccoons, and striped skunk. Also, Perez et al (2012) reported root damage destroying some eggs or nests from beach grasses. Although as a species terrapin have evolved to survive the normal risks associated with living in an estuary, the increased stress associated with anthropogenic sources may be sufficiently high enough to cause local extinction (Roosenburg, 1990). In summary, there is a lack of knowledge of the terrapin's life history parameters including age and sex specific population structure, fecundity, growth rates, and mortality rates.

Terrapins exhibit sexual dimorphism and are oviparous (Seigel, 1984). The females have a larger body and head, and travel farther distances, mostly for nesting (Seigel, 1984; Sheridan et al, 2010). In the first two years of their lives male and female terrapin grow similarly, but diverge in size after age three (Seigel, 1984). Size differences can be used to differentiate between adult males and females. The cloacal placement, keel size and head shape can also be used to differentiate between male and female terrapin. Normally terrapins will not move from their foraging area except during nesting season during which female terrapins will lay their eggs at specific nesting beaches (Pritchard, 1967; Davenport and Macedo, 1990). Terrapins repeatedly return to the same beach area to lay their eggs, and therefore are vulnerable to habitat fragmentation (Roosenburg, 1994). Sheridan et al (2010) reported terrapin moving distances greater than 8000 m from a nesting beach back to the marsh, and an average of 203 m between nesting sites. Coleman et al (2014) noted terrapin moving much farther to nesting sites in northern latitudes (>500m) than in southern latitudes (15m).

#### **Nesting Season**

Nesting season, and other attributes related to nesting vary with latitude (Palmer and Cordes, 1988). Exact dates for the terrapin nesting season in the Galveston Bay area are unknown although Hogan (2003) observed one terrapin nesting in April on South Deer Island. Before the nesting season terrapin can be observed moving into creeks, and bays for courtship and copulation (Palmer and Cordes, 1988). Copulation is initiated and completed in the water. Terrapin may nest as many as three times a season (Roosenburg, 1996). Feinberg and Burke (2003) observed terrapin nesting from April through July in New York. Seigel (1980) observed terrapins nesting from May to June in New Jersey.

Nesting season lengths vary from 34 days in New York, 57 days in Florida, and 60 days in South Carolina. Terrapin nest during both the day and night, and the females will cross through the marsh to get to their nesting site (Hogan, 2003). The nesting activity of many turtle species appears to be linked to the weather and climate (Bowen et al, 2005). Terrapin nesting activity in New York increased with daily high temperature and during high tides (Feinberg and Burke, 2003).

#### **Nesting Habitat**

Selection of nesting habitat has numerous effects on the demographics and survival of terrapin. Roosenburg (1996) recognized that habitat selection was primarily a maternal effect on the life history of an organism. Terrapin exhibit environmental sex determination which is driven primarily by ambient temperature. Ambient temperature during terrapin development in the egg can affect sex determination along with size, growth, behavior, and survivorship (Roosenburg, 1996). Species with environmental sex determination frequently produce clutches with skewed sex ratios, which is thought to be a mechanism to prevent inbreeding (Roosenburg, 1996). Inbreeding between siblings is prevented because the entire clutch is the same gender (Roosenburg, 1996). Warmer temperatures have been shown to produce females, while cooler temperatures produce males (Roosenburg, 1996). A couple of mechanisms have been suggested for maternal selection of nesting sites. One theory suggests female terrapin assess the current sex ratio and choose a site that would favor a particular gender (Roosenburg, 1996). This is highly unlikely because terrapin do not appear to have a way of assessing the current sex ratio. An alternative theory is more complex and suggests terrapin can sense the amount of

energy needed to develop an embryo through hatchling development (Roosenburg, 1996). Terrapin choose a site that would have the most benefit a particular clutch due to egg size (Roosenburg, 1996). He found that terrapin tend to lay smaller eggs in cooler (male producing) areas. These cooler areas are thought to be in shaded areas, but the depth of the nest, can also affect ambient temperature, which in turn influences the sex of the terrapin offspring (Roosenburg, 1996). Roosenburg, (1996) found that a 2-3 cm change in nest depth can drastically change the temperature of the incubating clutch (Roosenburg, 1996).

Terrapin from various locations have been shown to generally prefer sparsely vegetated, flat to gently sloping beaches for nesting (Seigel, 1980; Palmer and Cordes, 1988). Terrapins also exhibit a preference for nesting sites located near aquatic habitats, which suggests a connection between nesting and tidal movements (Seigel, 1980; Palmer and Cordes, 1988). The Texas Diamondback terrapin share many of the same nesting preferences with the northern terrapin because they are known to choose areas that are gently sloping, sparsely vegetated and above high tide (Hogan, 2003; Palmer and Cordes, 1988). Palmer and Cordes (1988) reported optimum nesting habitat suitability occurs at locations possessing 5% to 25% vegetation coverage, a mean slope of less than or equal to 7°, and above the high tide line. However, Montevecchi and Burger (1975) found that terrapin select nesting areas independent of vegetation cover. Roosenburg (1996) found that terrapin which nested in less vegetated areas produced females versus those which nested in more vegetated areas which produced male offspring. Roosenburg (1996) observed nesting sites in areas with full sun and no vegetation or areas on the edge of vegetation. Only a few nests were observed in the middle of highly vegetated areas

(Roosenburg, 1996). This could be due to the issues with root inundating the eggs or with nest excavation.

Terrapin from various locations along the Atlantic coast have been shown to generally prefer sandy areas for nesting (Seigel, 1980; Palmer and Cordes, 1988). In contrast, the Texas Diamondback terrapin differs from the northern subspecies in that it has been observed nesting in shell hash rather than sandy areas. Similarly to Texas Diamondback terrapin, the Mississippi Diamondback terrapin (Malaclemys terrapin *pileata*) are documented nesting on shell hash beaches (Coleman et al, 2014). Shell hash is primarily composed of different fragments from the shells of oysters and other mollusks, sediment, and live and dead plant life. The difference in substrates used by some subspecies of Gulf of Mexico and Atlantic subspecies of terrapin for nesting redefines the currently accepted nesting habitat requirements reported in the literature (Palmer and Cordes, 1988). Shell hash beaches can be found inside barrier islands, within the estuary where oyster reefs are located. These islands are distributed throughout the northern Gulf of Mexico. This nesting habitat may provide conditions that lead to the greatest survival of the terrapin hatchlings. The exact mechanism is unknown but certain features such as stable structure, thermal insulation and good drainage might be important factors. When these hatchlings grow to adults, they will likely return to the same nesting beach to lay their eggs (Sheridan et al, 2010).

#### **The Reproductive Process**

Copulation does not necessarily immediately precede egg fertilization, because females can store sperm. Some females are known to store sperm for up to 4 years, and

they can store sperm from multiple males (Hogan, 2003). Aggressive male competition or combat for females is apparently absent in terrapin (Seigel, 1984). Sperm storage is thought to occur due to asynchronous reproductive cycles in males and females (Girling, 2002). Asynchronous reproductive cycles are thought to reduce the risk of predation by decreasing copulation frequency, and act as insurance in finding a partner during times of low density or movement (Girling, 2002). Follicles are stored, develop, and fertilized in the oviduct (Girling, 2002). Due to the deficiency of studies on follicle development of the Texas Diamondback terrapin, estimates of developed follicle size are unknown but based on other similarly sized turtle species. A follicle is considered mature at a diameter of higher than 15mm (Ernst, 1971).

Vitellogenesis is the process of developing the yolk, in which the female terrapin secrets pituitary hormones and steroid hormones to regulate the uptake of vitellogenin by the oocyte (Callard and Ho, 1980). Females can be vulnerable at this time due to the extra energy uses during vitellogenesis. Callard et al (1978) observed a decrease in somatic fat deposition during vitellogenesis in some reptile species. Vitellogenin is distributed evenly to each mature follicle during yolk development, which is illustrated by the lack of variation in individuals per clutch (Roosenburg and Dunham, 1997).

After oviposition, unused follicles go through atresia in which the female reabsorbs the unused follicles. In some turtle species, follicular enlargement begins soon after oviposition of the last clutch near the middle or end of the summer season (Callard et al, 1978). Terrapin will begin looking for a suitable nesting site by sand sniffing. Lazell and Auger (1981) first observed terrapin sand sniffing and theorize terrapin are "sniffing" to avoid areas of high plant rhizome density. Once a suitable area site is located, they dig the nest by scooping out sand or shell hash with their back feet. The digging behavior is similar to that described for the green sea turtles (Burger, 1977). Terrapins lay their eggs in a triangular or flasked shaped nest in the sand, and some females may lay several clutches in a season (Palmer and Cordes, 1988).

Nest depth varies greatly from 2.5 inches to 7.5 inches across their range (Burger and Montevecchi, 1975). Terrapin eggs are symmetrical and possess a positive bicone<sup>3</sup> (Montevecchi and Burger, 1975). An egg is composed of yolk and albumim and the yolk of a terrapin egg is composed mostly of protein and lipid material. The composition of the terrapin's albumen is less understood, but is thought to be composed of water, nonpolar lipids, and lean dry mass (Roosenburg and Dennis, 2005).

Terrapin can multi-clutch<sup>2</sup> and some terrapin have been documented nesting several times in one season. Large females tend to lay the most eggs (Palmer and Cordes, 1988). Clutch sizes will vary from 4 to 18 eggs, and the clutch size varies with latitude. Terrapin nesting in lower latitudes usually produce fewer but larger eggs than females in the north (Palmer and Cordes, 1988). Roosenburg et al. (2005) suggested that the longer growing season of the southern region increases temperature-dependent energy consumption, and the larger size is due to the higher lipid reserves the terrapin needs for survival.

<sup>&</sup>lt;sup>2</sup> Multi-clutch – an individual terrapin laying multiple clutches of eggs during one nesting season

<sup>&</sup>lt;sup>3</sup> Positive bicone – denotes a symmetrical eggs with blunt ends

The length and width of terrapin eggs vary with latitude. For example terrapin eggs in Texas and Florida exhibit an average length of 3.9 cm and average widths of 2.3cm and 2.23 cm, respectively (Hogan, 2003; Seigel, 1980). In contrast terrapin eggs from New Jersey and Maryland have been reported to have average lengths and widths of 3.11to 3.48cm, and 2.12cm respectively (Palmer and Cordes, 1988; Roosenburg and Dennis, 2005). In addition, to the higher temperature – larger egg hypothesis there are other theories that have been proposed to explain this gradient. The different theories of factors affecting egg length involve carapace length, resource availability, female pelvic aperture and optimal egg size theory (Roosenburg and Dennis, 2005). Optimal egg size theory states that a terrapin egg will grow to a certain size regardless of the mother's body size, or other morphological factors (Roosenburg and Dennis, 2005). In the traditional optimal egg size theory, egg size is determined by selection for traits that produce the greatest number of surviving progeny possessing the greatest fitness (Roosenburg and Dunham, 1997). In summary, the female's energy is directed not to larger eggs, but to larger clutch sizes. Therefore, resources will affect the clutch size and not individual egg length. Montevecchi and Burger (1975) research appears to support the optimal eggs size theory because they found no correlation with plastron length and egg size or shape. Also, Montevecchi and Burger (1975) found a positive correlation with plastron length and clutch size. An additional study by Ernst (1971) failed to find any correlation between ovarian weight and plastron size.

Incubation periods vary with temperature and can range from 61 to 104 days (Palmer and Cordes, 1988). The survivorship of nests in the Chesapeake area was estimated at 1-3% depending on environmental conditions (Roosenburg, 1991).

Terrapin hatchlings do not crawl to the water as many sea turtle do, but are usually observed heading up the beach and away from the open water (Butler et al, 2004; Coleman et al, 2014). Roosenburg et al (1999) reported hatchlings moving into heavily vegetated near shore areas after birth. Terrapin released in open water swim to the cover of the shoreline or to the tidal rack (Mitro, 2003). In Texas, captured young terrapin immediately seek heavily vegetated areas when released (Guillen – pers. comm). Mississippi terrapin hatchlings showed preference toward heavily vegetated marsh when released (Coleman et al, 2014). The first few years of the terrapin's life is known as the "lost years" due to the hatchlings' disappearance into heavily vegetated areas and their apparent cryptic behavior. Female terrapin will reach sexual maturity around 8 to 13 years, and male terrapin reach maturity at 4-7 years, depending on range (Roosenburg, 1991).

Understanding reproductive activities is critical for estimation of population size and demographics which is needed for development of useful conservation management regulations (Mitro, 2003). Follicular data can be used to characterize the timing of follicular and egg development. This is important because during vitellogenesis terrapin are expending energy by producing pituitary and steroid hormones to control the development of yolk. Female terrapin are especially vulnerable at this time to any disturbances especially from anthropogenic sources. For example, destruction of critical nesting habitat could mean the death of the terrapin and her offspring because she does not have the extra energy needed to relocate, or deal with the higher levels of stress. Timing of reproductive events, especially early or late in the season, influences overall fecundity and survival of both adult and hatchlings (Bowen et al, 2005). The

understanding of the timing of these life-history events is critical information used in conserving terrapin.

Loss of the terrapin's nesting beaches can have multiple devastating effects on the population. First, terrapin will waste energy and could very likely be injured trying to return to their nesting beach (Sheridan et al, 2010). Loss of particular nesting areas can lead to altered sex ratios, if the beaches with microclimate favoring one sex are lost or the degraded nesting habitat will reduce the already low hatchling success (Roosenburg, 1991). If a terrapin is not able to find an alternative nesting location then dystocia can occur (Sheridan et al, 2010). Dystocia, egg-binding, will force the eggs to be retained in the oviduct and the eggs can move into the abdominal cavity (Sheridan et al, 2010). Eggs in the abdominal cavity are at high risk of bacterial infection that can lead to the death of the affected terrapin (Sheridan et al, 2010). Therefore, human construction and channelization activities which can result in loss of nesting beaches should be carefully evaluated since irreversible negative impacts on terrapin can result (Roosenburg, 1991).

#### **Previous Studies of Terrapin in Texas**

Although considerable data exists on the east coast Diamondback Terrapin, little data exists on the Gulf of Mexico subspecies and in particular the Texas Diamondback terrapin, *Malaclemys terrapin littoralis*. Extant populations of terrapins have been found in Galveston Bay, Sabine Lake, and Nueces Bay (Koza, 2006; Glenos, 2013). Past studies have documented a distinct population of terrapin on South Deer Island (Hogan, 2003; Haskett, 2011; Glenos, 2013). South Deer Island is approximate 0.3 square kilometers, and major predators such as coyotes and raccoons appear to be absent. Muddy saltmarsh substrate is dominated by large stands of *Spartina alterniflora*, *Batis maritima* and other marsh plants are found throughout South Deer Island. Based on informal surveys, there also appears to be abundant prey on South Deer Island, including marsh periwinkle, *Littorina irrorata*. Roosenburg (1991) and Roosenburg et al. (1999) noted that terrapin populations in their studies were found in habitats similar to the South Deer Island population. The Environmental Institute of Houston (EIH) has ongoing studies on the Texas Diamondback terrapin and has discovered an established population on South Deer Island and surrounding areas (Glenos, 2013; Haskett 2011). Hogan (2003) conducted surveys of shell beaches on South Deer Island from April 2001 to May 2002. These beaches were checked twice a day. Only one nesting terrapin was found and this terrapin is believed to be the first documented terrapin nesting in Texas (Hogan, 2003). A picture<sup>4</sup> and a GPS point was provided to EIH of terrapin nesting activity on Shell Island during 2012. Also, possible terrapin scrapes were observed near the pitfall traps on June 6, 2013 during this study (Figure 1).



Figure 1. Possible terrapin scrape.

Studying nesting terrapin in their southernmost range is much more difficult than in the north due to limited access to potential nesting sites. Many of their potential nesting sites are located on small isolated barrier islands (Borden and Langfords, 2008). They often share this nesting habitat with sensitive and government protected shorebird and colonial waterbird species. The habitat shared by terrapin and protected shorebirds creates logistical and legal issues when trying to study terrapin nesting due to restricted access.

As previously mentioned, terrapin in their northern range, along the Atlantic coast, nest on sand beaches. While walking on sand, terrapin leave tracks which are used by researchers to find the terrapin's nest. Butler et al (2004) reported that following female terrapin tracks was the most reliable method of locating their nests. Consequently, researchers in the terrapin's northern range are able to perform daily nesting surveys on beaches that have easy access. In contrast, shell hash does not leave clear signs of nest digging or terrapin tracks. The lack of clearly recognizable tracks has made finding terrapin nesting areas in Texas extremely difficult. Researchers studying the Mississippi terrapin have similar issues with hidden terrapin nests (Coleman et al, 2014).

#### Objective

The objective of the current study was to determine 1) when do terrapin nest in Galveston Bay and 2) what physical attributes are associated with areas where terrapin have historically nested. To answer the first question, a portable ultrasound was used to collect follicular data. Ultrasonography is a non-invasive approach that can be used to determine the reproductive stage of a specimen under field conditions and has been a

proven technique in determining follicular activity in other marine and freshwater turtle species (Lance et al, 2009; Robeck et al, 1990). Previous techniques including celioscopy, and laparoscopy were very invasive and there was a high risk of specimen death (Pease et al, 2010). Nesting habitat data was collected at locations where terrapin have been previously observed nesting. There were four variables measured during the nesting habitat surveys. These include: 1) shell hash zone width, 2) elevation, 3) vegetation beyond shell hash zone, 4) and sediment composition. Nesting habitat data was indirectly measured using pitfall traps to answer the second question regarding where terrapin nest. This information would be useful in the future development of predictive habitat suitability models that use habitat variables to predict overall probability of successful terrapin nesting.

# Methods

# **Study Site**

Female terrapin from 6 different sites were examined for follicular development, but only 2 of these sites were used for the pitfall trap deployment and habitat surveys. The six sites are Greens Lake, Bolivar Peninsula, South Deer Island, North Deer Island, Sportsmans Road and Shell Island (Figure 2). Each site was chosen because it appeared to have the proper habitat as defined in the literature. Shell Island and South Deer Island were chosen for pitfall trap deployment and habitat surveys. These two areas were chosen for the additional data collecting because terrapin nesting activity had been previously documented at these locations.



Figure 2. Galveston Bay with all sites marked.

Terrapin have been previously documented at all six chosen sites and all sites have a variety of marsh plants, ample prey available, and extensive creeks for terrapin survival (Glenos, 2013; Clarkson, 2012; Haskett, 2011). North Deer Island is a small island owned by the Audubon society, and Sportsmans in close proximity on the main island of Galveston. Greens Lake is a sub bay of Galveston Bay and Bolivar flats is a sub bay of East Bay. Shell Island is a 1.21 kilometer peninsula consisting primarily of shell hash located in Texas City (Figure 3). It extends into Dickinson Bay and it is owned by the Nature Conservancy. South Deer Island is a 0.3 km square Island in Galveston bay (

Figure 4) (Hogan, 2003). Shell Island and South Deer Island are the only sites with previous terrapin nesting documented.



Figure 4. South Deer Island.



Figure 5. Bolivar Flats.



Figure 6. Greens Lake.



Figure 7. North Deer Island.



Figure 8. Sportsmans Road.

#### **Environmental Assessment**

Prior to searching for terrapin an environmental quality assessment was conducted. The assessment included air and water temperature, wind speed, turbidity, salinity and cloud cover. Water temperature (°C), was measured with a thermometer left in the water for at least one minute. Air temperature (°C) was measured with a Kestrel 3000 Wind and Weather Meter in the shade. The Kestrel instrument was also used to measure average wind speed (mph) and wind direction using a compass. Water turbidity was measured with a Secchi tube. The Secchi tube was placed in the shade and read. Percent cloud cover was estimated by sight. Salinity was measured with an Extech RF20 refractometer looking into the sun with water on the lens but keeping the refractometer level after zeroing the scale with tap water. Also, the time and location of arrival at the collection site was determined with a GPS and watch, and recorded.

#### **Capture Methods**

Four methods were utilized to collect terrapin. These include modified crab traps, radio tracking, pitfall traps, and timed walking searches. The modified crab trap, time walking searches, and radio-tracking were used primarily to collect non-nesting females and male terrapin in areas where colonial waterbirds were not nesting to assess demographic composition of the terrapin in the vicinity of the nesting beaches. General terrapin surveys (land searches and modified crab traps) were conducted each week in addition to deploying pitfall traps, and continued after the pitfall surveys were completed. The pitfall traps were used to target the collection of nesting terrapin. All activities

conducted during this study were done under an approved animal care protocol (IUCAC # 10.005).

### **Crab Traps**

The traps used are the typical four door, wire, blue crab traps with a modified chimey that allows terrapin to breath while on the trap. Fresh baits purchased from shrimp trawlers including shad, menhaden and mullet were used. The minimum amount of time and number traps fished were 2 hours and 3 traps.

#### **Land Searches**

Walking land searches were conducted for a minimum of two hours per surveyor in a randomly selected transect of a specified area of the adjacent wetland when possible. At least two surveyors were utilized for a total minimum time of 4 man-hours of search time. Land searches of selected areas were restricted or terminated if nesting colonial waterfowl were present. Search time was halted if a terrapin was captured to allow time to process the terrapin.

### **Radio Tracking**

South Deer Island, North Deer Island and Sportsmans Road had terrapin tagged with Advance Telemetry System. Inc. (ATS) radio tags. When these areas were surveyed, the tagged terrapin would be tracked along with transect searches. While the researcher was walking their transect they were also scanning for tagged terrapin with an

ATS scientific receiver and 3 element folding Yagi. When a signal was recognized, transect search time was halted and the radio tracking time was initiated and recorded. Radio tracked females were checked for reproductive stage, habitat was recorded and morphometric data was collected if the previous capture exceeded six months.

#### **Pitfall Traps - Nesting Terrapin**

The other part of my study involved the use of pitfall traps and reproductive state monitoring with an ultrasound machine. The pitfall traps were used to catch gravid female preparing to nest and the collection of valuable morphometric data. The pitfall traps also helped reduce another problem, which is stress on adjacent colonies of nesting birds. We did not want to disturb or stress the waterbird colonies during their nesting season. The use of pitfall traps along the periphery of the island reduced the need to walk further into the wetlands searching for terrapin during this period.

The capture method that was used to monitor nesting terrapin activity during this experiment was modeled after Borden and Langford's (2008) simple pitfall traps. First, an appropriate length of drift fences was buried to a depth of 30 cm along the beaches of South Deer Island, and Shell Island. One of the silt fences was placed along Shell Island's beach, and three fences on South Deer Island's beach. The exact placement of the silt fences and pitfall trap was determined by suitable nesting habitat, such as high exposure to the sun, protection from water inundation, low-slope, and shell hash (Hogan, 2003). The pitfall traps were constructed from 19 gallon plastic storage containers. The lid was modified to rotate so the terrapin had coverage after capture. There was one pitfall trap in the middle of the fence and one at each end of the fence. When the traps
were not in use chicken wire was placed over them to prevent terrapins from being captured in un-checked traps. Traps were checked daily for one week each month from March 2013 to June 2013

## Environmental and Biological Data Collected at Time of Capture

When a terrapin was found, before the surrounding habitat was disturbed, a GPS reading was taken to mark the point a capture. Using an infrared thermometer, the temperature was taken of the carapace top and the soil temperature. The percent vegetation, dominant vegetation, and dominant vegetation height within a 1 meter area surrounding the point of capture was recorded. If the terrapin was found in water, the water temperature, creek width, and creek depth were recorded. Next, the terrapin's initial behavior was recorded such as sitting, walking, swimming, or buried in the mud. The terrapin was examined for distinct notches of its carapace to determine if it had been previously captured, marked and released. The Cagle notching system was used to provide a unique number for each terrapin (Cagle, 1939). An Avid Minitracker I PIT tag reader was used to determine if the terrapin had been previously tagged with a PIT tag. When a terrapin was captured, several morphometric measurements were taken, in units of millimeters, to monitor and estimate growth. These measurements were conducted using large calipers. Measurements included: terrapin's length (notch to tip and 1st marginal scute to tip), terrapin's width (second suture and max length), terrapin's depth (second suture and second keel), terrapins' head width, and terrapin's plastron length and width (Figure 9).



Figure 9. Labeled terrapin for aid in measurement.

The terrapin was also weighed, and assessed for injuries. If the terrapin lacked a notch mark and/or PIT tag they were marked with appropriate notches and given a 12 mm Avid PIT tag. The PIT tag was injected in the left posterior leg. The area was sanitized with isopropyl alcohol before the injection and new skin was placed on the injection site after removing the needle. Photos of the terrapin were taken, and its behavior was recorded upon release. Body condition was calculated with Fulton's equation (Caldarone et al, 2012) using carapace midline and weight:

Body Condition = Weight (g) / Length<sup>3</sup> (mm) \* 100,000

When a female terrapin was captured additional information of reproductive stage was taken throughout the year. Identifying information such as notch number and PIT tag were electronically recorded along with acoustic imagery using a Sonosite<sup>®</sup> 180 Plus Ultrasound (Sonosite Inc., Bothell, Washington, USA,Figure 10) so pictures could be

matched to individual terrapin. Ultrasonography is a low risk technique used to evaluate the internal organs and status of unborn young of a variety of species including turtles, canines, bovine, and even humans (Wilkinson et al, 1990). First, the terrapin was palpated by placing fingers below the lower bridge and above the leg joint on both sides of the terrapin. The researcher applied only gentle force so to not hurt the terrapin or her potential eggs. Then, the female terrapin was placed in dorsal recumbency and her back leg was held out to make room for the ultrasound probe. The area was coated with VetImaging<sup>®</sup> veterinary formula ultrasound gel and the probe was placed in between the leg and lower shell bridge. Pictures were taken of the follicular development and follicles and eggs were measured with the virtual software calipers (Figure 11). Both sides of the terrapin were checked for follicular development. Then the stage of reproductive development was recorded and the terrapin was released. There was some repetition in reproductive stage check and follicle measurements from a single terrapin at different times in the season. Sonosite<sup>®</sup> imaging software was used to download the pictures from the ultrasound machine. Egg and follicle measurements were further analyzed and checked for accuracy later in the lab with ImageJ<sup>®</sup> software (Figure 11). Only the largest follicle measurement was recorded. Since ultrasound, unlike x-ray, is incapable of detecting and imaging all follicles present, I did not provide measurements of other follicles observed.



Figure 10. Ultrasound machine with coupling gel and female terrapin.



Figure 11. Examples of output from ultrasound. A= Egg, B= ImageJ follicle measurement, C= Follicle without measurement, D= Follicle with measurement from internal calipers.

### Data Collected from Potentially Nesting Terrapin Captured in Pitfall Traps

When a terrapin is pulled from a pitfall trap, the morphometric measurements, and ultrasound data previously described were taken. Basic information, such as location, tide stage, and weather, were recorded. Any bycatch in the pitfall traps was recorded and carefully released. After a terrapin was released, it was visually monitored for a minimum of 15 minutes for signs of nesting. If a terrapin nested, the nest was marked with four stakes and orange flagging.

#### **Nesting Habitat Surveys**

Habitat data was be used to identify areas with a high potential for terrapin nesting using the following variables: shell hash zone width, elevation, vegetation beyond shell hash, and sediment composition. Shell hash zone width was chosen as an important variable because it could play a part in protecting the eggs from erosion and inundation by providing more layers of protection from water and waves. Elevation was chosen because previous research has documented terrapin choosing areas of high elevation to avoid risk of egg mortality due to inundation by rising water (Hogan, 2003; Seigel, 1980; Palmer and Cordes 1988). Vegetation beyond shell hash was chosen because terrapin are known to move toward the vegetation to avoid predation and seek out suitable prey and environmental conditions. Sediment size and composition was chosen because these traits may affect the ability of female terrapin to dig a nest and because the sediment type can affect water drainage and temperature which influences sex determination of the young and mortality rates. Hatchlings have also been documented burying themselves in the shell hash to prevent desiccation (Coleman et al, 2014).

Shell hash availability and elevation were collected with a Sokkia Total Station Set 330R and target prism (Figure 12). The target prism was setup by adjusting it to the desired height and placing one prism in the correct place. Then, total station was leveled by adjusting the height. After the station was level, the station was set to magnetic north and then corrected for the magnetic declination (previously calculated). Next, the northing and easting the coordinates were inputted. Northing and easting are used because the areas were mapped in Universal Transverse Mercator (UTM) projected coordinate system, which is best for small areas. Finally, the target prism (bottom to middle of prism) and total station's (bottom to line marked on station) heights were recorded in the total station. After testing the setup, the total station and prism were ready to record. As the target prism was moved around the study area, the water line and changes in water level elevation were recorded with the total station. The usual total station method was to measure distances and elevations along a transect starting with the water line up to the top of the shell hash, and the end of the shell hash (Figure 13). Any rapid changes in direction or slope were also recorded by taking additional survey points needed to visually illustrate significant changes in elevation and slope. Obstacles and percent vegetation beyond the shell hash were noted.

Sediment cores were taken in areas that visibly appeared to have different shell composition or different sources of shell hash. The sediment cores were 5 inches (12.7 cm) deep and were brought back to the lab to be separated into size fractions. The sediment was separated with 3 different sieve sizes (3.2, 12.7 and 25.4 mm), baked at 105°C for an hour to evaporate all water, and weighed for percent composition.



Figure 12. Sokkia 330R Total Station (left) and target prism (right).



Figure 13. Identified points taken with the total station.

#### Data Analysis

### **Geospatial Analysis**

Data from each point was recorded in the field and entered into an Excel spreadsheet database. In the habitat Excel file any mistakes made in the field were corrected and the elevation (Z) was adjusted using concurrent tidal information obtained from a NOAA tide gage. Tide information for Shell Island elevation changes was obtained from NOAA's Eagle point tide gauge (Station ID: 8771013). The time zone was LST/LDT, the datum was Mean Sea Level, and unit used was meters. Tide information for South Deer Island was taken from NOAA's Galveston Railroad Bridge, TX (Station ID: 8771486). The total station recorded Z (elevation) for each point from the station at 10 meter (set as default). The Z of the points taken at the water line were corrected using the tidal gauge Zs and the correction factor was used to correct the rest of the Z points.

Using ArcMap 10.2, a map was made illustrating the spatial extent of four habitat variables: shell hash zone width, elevation, vegetation density beyond shell hash, and sediment composition. The values of habitat variables near the pitfall traps were used to define the "preferred" nesting habitat as defined by these four variables because these areas have the highest probability of terrapin nesting based on past evidence collected in Galveston Bay. Evidence of terrapin nesting included historical documentation (e.g. picture and/or GPS coordinates of a terrapin nesting), terrapin caught in pitfall traps during this study, or terrapin scrapes seen in area during this study.

Shell hash availability was spatially defined using the ArcMap measuring tool. The measurement was taken from the top of shell point to the end of shell hash point. The width of the shell hash zone above the water line was separated into three categories: low (1m - 3m), medium (4m- 7m) and high (8m-14m) by dividing the range of possible zone widths using the ArcGIS Jenks classification algorithm. Jenks classification breaks classes into similar groups and maximizes the variance between classes while minimizing the variance within classes (Wade and Sommer, 2006). To facilitate comparisons between sites, the width categories for South Deer Island were manually changed to match Shell Island's categories.

Reported elevation was based on mean sea levels and was digitized by using the Z factor in the Triangular Irregular Networks (TIN) tool. Elevation was separated into three categories: low (-0.3348m – 0.0339m), medium (0.0339m - 0.3255m) and high (above 0.3255m) by dividing the range of elevation using ArcGIS Jenks categorizing method for South Deer Island. Then, Shell Island elevation classes were manually changed to match South Deer Island's elevation categories.

Vegetation beyond shell hash was recorded as low (0 to 49% vegetation cover), medium (50 to 74 % vegetation cover) or high (75 to 100% vegetation cover) by sight in the field and compared to infrared imagery of vegetation from the 2004 National Agriculture Imagery Program (NAIP) for the area surveyed. The Iso Cluster tool in ArcGIS was used to group the infrared vegetation image into 25 or less classes by the intensity of the color in the image. The infrared groups previously identified were then validated using data from the field surveys and reclassified to the three levels listed above and applied to the entire area of interest.

The separated and weighed sediment size fractions were converted to ratios (weight of particle fraction ÷ weight of sum of all sediment fractions). Then, the

sediment sample ratios were displayed as pie graphs on the map in the areas where they were collected. The area around the pitfall trap on Shell Island was assumed to represent the best predictor of suitable nesting condition due to recent terrapin captured in the area, the previous confirmed and recorded nesting activity, and the observed nesting scrapes. The sediment composition measured at the Shell Island's pitfall area was used to determine areas of potential nesting habitat.

### **Data Analysis**

The largest follicle measurements were analyzed using the Minitab 16 statistical software package to determine if there was any relationship between follicle measurements and terrapin morphometric. Follicle measurements were tested for normality. Terrapin that did not have follicles present were not included in the analysis. Linear regression was used to determine if carapace length, weight, body condition and tide could be used to predict follicle size. Fitted line plots were used to illustrate correlation and possible predictive relationships. The lack of a significant linear relationship including the slope not varying from zero would support the optimal egg size theory which states that terrapin egg size is not influenced by any morphometric factors associated with the female terrapin under existing environmental conditions (Kern et al, 2013).

The relationship between body weight and follicle size was conducted by first taking the  $Log_{10}$  of weight and then running a linear regression, where x = log transformed weight and y= follicle measurement. The influence of weight, day of year and high tide (m) were tested using binary logistic regression where the binary variables

were absence or presence of follicles. Tide data was obtained from the Eagle Point station, and the mean high tide was recorded. Next, the models which exhibited statistically significant slopes were compared using  $R^2$  and the Akaike's Information Criteria (AIC) to select the statistical model with best predictive ability to detect changes and explain the greatest amount of variance in follicle size. The bias adjusted estimation equation used to calculate AIC (Burnham and Anderson, 2002) is depicted below:

$$AIC_{c} = n*ln (RSS/n) + 2*K + (2*K*(K+1)/(n-K-1))$$

Where  $AIC_c$  is the biased adjusted AIC estimator and n is number of data points, K is the number of parameters, and RSS is the residual sum of squares.

Monthly differences and trends in follicle size were monitored visually and evaluated using boxplots and 95% confidence interval plots for the median. Analysis of variance (ANOVA) and a post-hoc Tukey's multiple range test were used to test for differences in average follicle size between months.

# Results

# Habitat

The pitfall traps caught 4 terrapin (3 terrapins on Shell Island and 1 terrapin on South Deer). All four terrapin had eggs present when checked with an ultrasound. The pitfall traps were open for a total of 543 hour during the 25 days they were deployed. The catch per unit effort was 0.0074 terrapin/ hour.

The South Deer Island nesting habitat survey took 5 days and a total of 368 survey points were collected. The Shell Island nesting habitat survey took 2 days and a total of 152 survey points were recorded (Table 1).

Event	Date	Site	Comments	
Pitfall Trap	4/18/2013	Shell Island		
Pitfall Trap	5/28/2013 to 5/31/2013	Shell Island	1 terrapin collected (SI)	
Pitfall Trap	6/4/2013 to 6/7/2013	Shell Island, South Deer	2 terrapin collected (SI)	
Pitfall Trap	6/25/2013 to 6/28/2013	Shell Island, South Deer		
Pitfall Trap	7/9/2013 to 7/12/2013	Shell Island, South Deer		
Pitfall Trap	7/30/2013 to 8/2/2013	Shell Island, South Deer	1 terrapin collected (SD)	
Habitat Survey	12/4/2013	Shell Island	66 points collected	
Habitat Survey	12/5/2013	Shell Island	86 points collected	
Habitat Survey	12/16/2013	South Deer	84 points collected	
Habitat Survey	12/17/2013	South Deer	126 points collected	
Habitat Survey	12/18/2013	South Deer	50 points collected	
Habitat Survey	8/18/2014	South Deer	84 points collected	
Habitat Survey	8/19/2014	South Deer	24 points collected	

Table 1. Summary of nesting habitat surveys on South Deer and Shell Island.

On South Deer Island 87% of the surveyed area had shell hash of various widths that could be used by terrapin for nesting. In contrast, 40% of the area surveyed on Shell Island was shell hash of various widths that could be used by terrapin for nesting. The preferred nesting habitat was defined and based on the higher pitfall trap catches and historical data on Shell Island. The preferred area had a wide shell hash zone widths (6m -14m) as illustrated in Figure 14. Relatively high elevation (> 0.3255m) as illustrated in Figure 15 was also observed at this site. Furthermore it had medium to high amounts of

vegetation (Figure 16). Sediment composed of fractions consisting of mostly 25.4 mm to >12.7mm diameter shell hash particles, then 12.7 mm to >3.2 mm with smaller amounts of larger (>25.4 mm) and smaller (<3.2 mm) sediment fractions were encountered at this site (Figure 17).



Figure 14. Shell hash zone width of Shell Island. The area with the pitfall trap shows the preferred width of shell hash being used to define predicted nesting habitat.



Figure 15. Elevation of Shell Island. Pitfall trap marks the preferred elevation being used for the predicted nesting habitat.



Figure 16. Vegetation density classes of Shell Island beyond shell hash. Vegetation classes are defined as follows: Low- 0-49% vegetation cover, medium- 50-74% vegetation cover, and high- 75-100% vegetation cover. Vegetation classes of areas beyond the shell hash pitfall trap vicinity were used for the predicted nesting habitat vegetation requirements.



Figure 17. Sediment cores taken from Shell Island showing sediment composition. Sediment core near pitfall trap shows the sediment composition used for defining predicted nesting habitat characteristics.

The extent of the shell hash availability on South Deer Island is shown in Figure 18. The majority of shell hash zone widths observed were within the narrower (1m-3m) range. Also, the imagery shows areas without shell hash available. Shell hash zone width, elevation and sediment type were not measured in areas without shell hash on South Deer Island and were excluded from further analysis. South Deer Island's elevation profile of the shell hash zone is shown in Figure 19. South Deer's vegetation classes are shown by Figure 20. The majority of South Deer Island has acceptable vegetation classes (medium to high vegetation cover; 50% to 100% cover) for terrapin nesting based on the range of conditions encountered and predicted from the nesting habitat on Shell Island. The sediment core locations and size composition on South Deer Island is shown in Figure 21.



Figure 18. Shell hash zone width of South Deer Island.



Figure 19. Elevation of South Deer Island.



Figure 20. Vegetation classes of South Deer Island. Vegetation classes are defined as follows: Low- 0-49% vegetation cover, medium- 50-74% vegetation cover, and high- 75-100% vegetation cover.



Figure 21. Sediment cores and sediment size composition of South Deer Island.

Using some of the range of values for habitat variables previously measured near the pitfall traps on Shell Island, other predicted nesting areas were identified on Shell Island and South Deer Island. Only three of the variables were used (shell hash zone width, elevation, and vegetation) because sediment composition differed significantly between South Deer Island and Shell Island (Figure 22). Since terrapin nesting has been documented on South Deer Island, it is known that some range of the shell hash composition measured on South Deer Island is utilized as nesting substrate. However, based on the low pit trap catch rates (one terrapin) and the lack of specific coordinates for previously reported nesting activity observed by Hogan (2003), no specific location could be selected as "preferred" shell hash composition. For future analyses, the sediment size data was pooled from both survey areas (Figure 23). This data will also be archived for comparison to future observed nesting occurrences at South Deer Island, at which time the substrate preference relationship can be refined.

Potential nesting habitat on Shell Island is shown by Figure 24 and three areas were identified as having similar characteristics to known nesting areas located where pitfall traps were deployed on Shell Island. Potential nesting habitat on South Deer Island is shown by Figure 25, and seven sectors were identified to have high elevation (0.3724 to 0.7140m), high to medium shell hash zone width (4 -11m), and near medium to high density classes of vegetation levels.



Figure 22. Comparison of sediment size classes from Shell Island and South Deer Island. The green box indicates the 95% confidence interval for the median.



Figure 23. Comparison of sediment size classes from Shell Island and South Deer combined. The green box indicates the 95% confidence interval for the median.



Figure 24. Potential nesting sites on Shell Island.



Figure 25. Potential nesting sites on South Deer Island using variable ranges defined from pitfall trap areas at Shell Island.

## Follicle Data

A total of 143 terrapin from six sites were checked for reproductive stage and follicle growth including 1 terrapin at Bolivar, 3 terrapin at Shell Island, 7 terrapin at Greens Lake, 13 terrapin at North Deer Island, 101 terrapin at South Deer Island, and 18 terrapin at Sportsmans Road (Figure 2). The following reproductive stages were observed: 49 terrapin had no eggs or follicles present, 88 terrapin had only follicles present, 1 terrapin had only eggs present, and 5 terrapin had eggs and follicles present. The average maximum follicle measurement was 1.457 cm  $\pm$  0.3379 cm. The largest maximum follicle measurement observed was 2.11 cm and the smallest measurement detected was 0.75 cm (**Appendix 2:Data collected on individual captured terrapin.**).

Maximum follicle measurements were normally distributed. I failed to detect any significant relationship between follicular size and carapace length (p=0.225), weight (p=0.396), Log<sub>10</sub> weight (p=0.304), or body condition (p=0.962) (Figure 26, 27, 28, and 29). A one way ANOVA test detected significant differences between the average follicle size measurements of each site (p=0.001) (Figure 30). Bolivar was excluded from the analysis due to low sample size (n=1). A Tukey's multiple comparison test showed that terrapin from Greens Lake had statistically significant larger maximum follicle measurements than the other sites. Shell Island and South Deer had similar follicle sizes and North Deer and Sportsmans Road both had statistically significant smaller follicles sizes (Figure 31). I did detect a significant linear relationship between follicle size and tide (p=0.046), and day of year (p=0.000) (Figure 32, Figure 33). Tide is known to have

an effect on terrapin nesting within the day, but the mechanism for affecting follicle size needs to be further explored.

We failed to detect any significant relationship using binary logistic regression between follicle or egg presence and parental length (p=0.575), weight (p=0.998), body condition (p=0.871), site (p=0.303), or tide (p=0.861). We did detect a slightly significant relationship with day of year (p=0.050) (**Appendix 3: Minitab Read Outs**).



Figure 26. Fitted line plot of maximum follicle measurement and carapace midline Estimated follicle size (cm) = 0.8294 + 0.003183 Length Mid (mm) Carapace.



Figure 27. Fitted line plot comparing maximum follicle length to weight. Estimated follicle size (cm) = 1.273 + 0.1357 Weight (kg).



Figure 28. Fitted line plot comparing maximum follicle measurement to Log10 weight. Estimated follicle size (cm) = 1.406 + 0.4297 Log weight.



Figure 29. Fitted line plot comparing maximum follicle measurement to body condition. Estimated follicle size (cm) = 1.421 + 0.00089 Body condition. Body condition was previously defined as W (g)/ $L^3$  (mm) \*100000.



Figure 30. Box plot of maximum follicle measurements from each site. Box size proportional to sample size. Bolivar was excluded due to low sample size (n=1).



Figure 31. Comparison of means of maximum follicle measurements from each site with 95% confidence intervals. The pooled standard deviation was used to calculate the intervals.



Figure 32. Fitted line plot of maximum follicle measurement versus day of year estimated maximum follicle size (cm) = 1.684 - 0.001175 DOY.



Figure 33. Fitted line plot comparing high tide to maximum follicle measurements; Estimated maximum follicle measurement (cm) = 1.538 - 0.4120 tide hh (m).

Tide, day of year, and interaction between tide and day of year showed to be the best predictors of follicle size (Table 2). However, the variance explained by these models was low (< 15%).

Model	к	RSS	AICc	$\Delta_{i}$	Wi	r <sup>2</sup>
Tide DOY Tide*DOY	5	3.05515	-289.368	0	1	0.1439
DOY	3	8.6577	-201.104	88.26368	6.82E-20	0.138
Tide DOY	4	8.6065	-199.438	89.92962	2.97E-20	0.143
Tide	3	9.5326	-192.536	96.83157	9.4E-22	0.051

Table 2. Comparison of goodness of fit between models using AIC and r<sup>2</sup>.

The box plot of follicle size from 2012 to 2013 illustrates a seasonal pattern with a decrease in follicle size from August 2013 to October 2013 (Figure 34). ANOVA documented significant differences between monthly average maximum follicle measurements (p=0.002). Tukey's multiple range test showed January, March, and April exhibited the highest mean maximum follicle measurements, and were significantly different than the other months. The other months with the exception of October were not significantly different in mean maximum follicle measurement. October had the smallest mean maximum follicle measurement and was significantly different than the other groups (Figure 36).



Figure 34. Box plot of maximum follicle measurements from 2012 to 2013.



Figure 35. Boxplot comparing overall mean maximum follicle size to each month. Box size is proportional to sample size.



Figure 36. Mean plot with 95% confidence interval for the mean maximum follicle measurement for all sites. The pooled standard deviation was used to calculate the intervals.

# Discussion

### Nesting Habitat

Ideal terrapin nesting habitat should increase the probability of successful production of hatchling terrapin. Higher amounts of suitable nesting habitat increases the likelihood that a female will not be limited by this resource and therefore more females will be able to nest. If suitable, female terrapin will return to the particular nesting area until limited by space. The primary criteria and assumptions used in defining potential nesting habitat were 1) previous records of terrapin nesting and 2) the associated physical data measured near those sites. It is reasonable to expect that since terrapin nested in these areas the physical attributes of these sites should be suitable for nesting at other locations.

Based on previous records it appears that Texas terrapin are primarily nesting in exposed shell hash. There may be several reasons for this pattern. First, previous nesting of all Texas Diamondback terrapin have been documented in shell hash. The terrapin nesting at Shell Island was found in shell hash and Hogan (2003) searched areas of exposed shell hash for terrapin. Also, another Gulf coast subspecies, the Mississippi terrapin, has been documented to nest in shell hash (Coleman et al, 2014). Terrapin likely choose elevated shell hash over the marsh or sandy sediment along the Gulf coast because terrapin eggs cannot survive high levels of water inundation due to high water levels or heavy rainfall. Given the physical dimensions of shell hash, it exhibits a much higher drainage rate when compared to other common estuarine sediment types. This is likely a major factor that would increase hatchling survival during heavy rainfall events.

Terrapin may also prefer shell hash over other areas with higher elevations for other reasons. I found that the higher elevation areas located at South Deer Island and Shell Island have some of the highest plant density. Rhizomes of actively growing plants are known to kill terrapin eggs and it is theorized that terrapin will scrape and sniff the sand to avoid these rhizomes (Lazell and Auger, 1981). Also, digging through areas with high rhizomes would be very difficult for terrapin and demand high amounts of energy. In addition, at South Deer Island, there were usually large congregations of laughing gulls nesting in and near the vegetated highlands. Laughing gulls are known predators of terrapin eggs (Brennessel, 2006).

Another potential reason for preferring shell hash over other substrates along the Gulf coast may be related to temperature sex dependency. Along the Atlantic coast, Roosenburg (1996) documented terrapin choosing vegetation edge sites for male clutches and open sun sites for female clutches. At my sites, the highland areas were highly vegetated and did not provide much thermal variation due to lack of variation in open canopy that is shaded versus open sunny spots. However, a 2- 3 cm change in shell hash depth can alter ambient temperature sufficiently to produce a mixed ratio of male and female hatchlings. The digging of nests in shell hash to different depths would increase the thermal variability of developing terrapin eggs and therefore reduce the likelihood of producing only one sex of offspring per generation.

Using multiple criteria I defined potential nesting sites for both Shell Island and South Deer Island (Figure 24 and 25). The process I used to develop the final criteria was based on multiple iterations using data from both habitat survey sites. Elevation and shell hash zone width from South Deer Island and Shell Island, when initially categorized
using the default spatial Jenks model output were not directly comparable. Consequently, I changed the Shell Island's elevation categories to match South Deer Island's elevation classes. Conversely I changed South Deer Island's shell hash zone width classes to match Shell Island's shell hash zone width categories. There was no a priori reason to pick one category system over the other in terms of boundaries of each category. Potential consequences of altering these class intervals are difficult to predict given the lack of recent terrapin nesting observations. Sediment size composition was not similar between sites (Figure 22). Due to the very different shell hash size distribution and composition between sites, I did not use this variable in the development of a final classification scheme, since using sediment particle size would result in no area on South Deer Island being classified as potential nesting habitat. However, nesting is known to occur on South Deer Island as documented by previous studies and observations. Shell Island had higher percentages of larger shell and South Deer Island had higher percentages of smaller shell. The range of sediment shows the variety of shell sizes terrapin are thought to nest, which still could be used to define sediment size and type "boundaries" to nesting habitat. Also, errors in calculating percent sediment size composition arose due to sampling methodology. Shell Island's southernmost core had high levels of clay. While sorting the shell hash a large amount of this clay was washed away. Washing the clay away affected the total sample size and skewed the distribution of the resulting sediment size. Therefore the southernmost core at Shell Island did not provide an unbiased estimate of the true distribution of sediment size at that area. Another source of potential bias is the sampling device. We found that the 0.15 meter diameter PVC pipe used to collect the sediment could have excluded the larger size fractions (e.g. larger whole shells) from the sample.

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The asymmetrical geometry of oyster shells in some cases would result in a negative bias against the inclusion of long narrow shells. Also, during the sampling process, some smaller sediment was lost by sticking to the sides of the sieves but this was likely insignificant. Sediment composition likely plays a major role in terrapin nesting site selection and hatchling survival, but it is unlikely sediment size and size composition alone could be used as a site selection criterion for Galveston Bay or the Gulf Coast. Other factors influencing nesting habitat selection and survival include predator presence, accessibility, and other physical factors measured during the study.

#### **Nesting Season**

The lack of correlation between follicle size and carapace length, weight, or body condition supports the optimal egg size theory. The optimal egg size theory states that turtles would not expend excess energy into egg size but instead into clutch size, therefore egg size should be similar between members of the same species under similar environmental conditions. However, the ultrasound technology used cannot accurately display the maximum number of follicles or eggs in a terrapin, due to the size of the terrapin, and the placement of the eggs. The available data are however sufficient to support the optimal egg size theory and my hypothesis that follicle development can be used with other factors to define the nesting season. The box plot of follicle development of 2012 to 2013 (Figure 34) documented a decreasing trend in 2013, but there was also lots of variation and a lack of measurements in 2012. The variation in average monthly follicle measurements maybe due to multi-clutching, multiple measurements of a single terrapin or the inability to recognize atretic follicles. Before a terrapin lays her first clutch of the season she has developing follicles for the next clutch. The follicle

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measurements used in the analysis were the largest follicles to show a pattern of development. Due to the variety of follicles sizes resulting from multi-clutching I did not observe a strong clear pattern of seasonal follicle size. Attretic follicles are being absorbed and if they are incorrectly classified it would lead to the false conclusion that an extended nesting season is occurring. Within seasons, sites that exhibited differences in average follicle size were most likely due to sample size and timing artifacts. For example, Greens Lake was not sampled as much as the other sites and five of the seven measurements were done during nesting season (May and June). Overall terrapin follicle size at the Greens Lake and Bolivar sites were larger in comparison to other sites. In contrast, North Deer Island and Sportsmans Road terrapin exhibited the smallest follicle measurements as a group, while South Deer Island terrapin exhibited intermediate size follicles. However these differences may be and artifact of limited sample size rather than true differences in population (Figure 30).

Based on the data collected I estimate that late April to early May is the most likely beginning of the nesting season. This is due to multiple reasons. First, seasonal temperatures were warmer which physiologically enables terrapin movement. Significantly larger follicle measurements were observed in January, March and April. However, many terrapin were not observed nesting at this time due to low seasonal temperatures. Many terrapin were observed aestivating through early March. Also, follicles need time to develop into eggs prior to oviposition. The second line of evidence was the timing of developed eggs. Terrapin with eggs were captured in pitfall traps on 5/30/2013, 6/5/2013, 6/6/2013 and 6/26/2013. Finally, Hogan (2003) reported nesting terrapin in April on South Deer Island. Nesting only lasts about 60 days in Florida and South Carolina. Texas terrapin should exhibit similar nesting season timing and length as northern and central Florida Gulf populations since many of the locations where terrapin exist are located at similar latitudes and exhibit similar seasonal temperatures. Based on these lines of evidence including 1) length of nesting season in similar areas, 2) the dates when terrapin were caught in the pitfall traps, and 3) the seasonal decline in follicle sizes it appears that June and early July would be the most plausible end of the nesting season. Follicle size declined in 2013 after June but August and September were not significantly different based on results of the ANOVA. This may be a false positive artifact associated with measuring atretic follicles. The best evidence for defining the nesting season would be direct observation of a decline in nesting individuals. However, due to their secretive behavior and limited access to the location of nesting we were not able to document actual nesting.

#### Conclusion

I conclude that the optimal egg size theory is supported by a lack of significant statistical relationship between follicle size and weight, length, and body condition. Terrapin nesting season in Texas likely extends from late April to early July based on a decline in follicle size, time periods when nesting terrapin were caught, and when scrapes were seen. Texas's nesting season is most likely similar to the nesting season in central and northern Florida based on similar climate. Based on examination of areas where terrapin nesting has been observed in the past the following traits seem to be key features associated with terrapin nesting habitat. These include shell hash areas with higher elevations (0.3255 m above MSL), medium to high widths of shell hash (6m to 14m) and medium to high densities (50 to 100%) of wetland vegetation in adjacent wetlands.

Terrapin are possible keystone species, which possibly regulate the density of periwinkle snails which can actually forage on live *Spartina* at high densities. Reproduction is crucial to the survival of a species and understanding the reproductive process, including identification of nesting periods and requirements, is needed in order to implement appropriate management practices to protect the species. Protection of critical shell hash nesting habitat is essential for the survival of this species within Texas.

#### **Future Research**

Additional research is needed to understand the entire life history of the Texas Diamondback terrapin. A critical ongoing information need is to actually find nesting terrapins throughout its range in Texas. This information along with the findings of this study would provide critical data needed to define nesting habitat variable s and season along the Texas and western portion of the Gulf Coast. Currently, research is being conducted by EIH to find the terrapin by locating areas and times terrapin are most likely to nest. In the future, researchers can search for nesting terrapin more deliberately using the nesting season and habitat qualifications established by this study. Once time and location of potential nesting is defined a combination of techniques can be used to document terrapin nesting. Pitfall traps, game camera, and boat surveys can be used on their own or together to locate and document nesting terrapin.

Additional habitat attributes should be evaluated as potential variables that influence terrapin nesting and hatchling success. For example, the amount, density, and

distance of wetland vegetation found beyond shell hash nesting areas and influence on hatchling behavior and success should be further evaluated and defined in futures studies.

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## Appendix

Date	Notch #	ID	Location	Latitude	Longitude	Tide
3/26/2012	186	186.037 813 077	South Deer	29.27372	-94.91200	0.044
3/26/2012	191	191.037 819 024	South Deer	29.27129	-94.91290	0.044
4/4/2012	167	167.037 819 000	South Deer	29.27429	-94.91312	0.348
4/4/2012	115	115.021 033 090	South Deer	29.27425	-94.91193	0.348
4/23/2012	618	618.070 586 363	South Deer	29.27025	-94.91200	0.086
4/23/2012	698	698.070 599 337	South Deer	29.27097	-94.91415	0.086
4/30/2012	705	705.070 784 582	South Deer	29.27041	-94.91250	0.17
5/7/2012	707	707.070 576284	South Deer	29.26987	-94.90955	0.23
5/7/2012	708	708.070 609 793	South Deer	29.51388	-94.53760	0.23
6/6/2012	173	173.037 841 054	South Deer	29.27037	-94.91087	0.109
6/6/2012	618	618.070 586 363	South Deer	29.27036	-94.91083	.109
8/1/2012	168	168.037 031 548	South Deer	29.27426	-94.91134	0.095
8/1/2012	320	320.048 595 258	South Deer	29.27426	-94.91134	0.095
8/1/2012	695	695.070 615 351	South Deer	29.27439	-94.91241	0.095
8/8/2012	604	604.070 591 020	South Deer	29.27363	-94.91199	0.14
8/8/2012	152	152.021 045 612	South Deer	29.27082	-94.91294	0.14
8/16/2012	727	727.070 586 861	South Deer	29.28431	-94.92091	0.126
8/16/2012	532	532.070 809 115	South Deer	29.27085	-94.91152	0.126
8/16/2012	101	101.021 032 110	South Deer	29.26989	-94.91155	0.126
8/23/2012	729	729.070 582 268	South Deer	29.26989	-94.91119	0.268
8/29/2012	732	732.070 597 840	South Deer	29.27337	-94.91083	-0.033
8/29/2012	56	56.015 824 526	South Deer	29.26985	-94.91299	-0.033
9/5/2012	392	392.057 821 114	South Deer	29.27327	-94.90997	0.066
9/5/2012	760	760.070 583 517	North Deer	29.28411	-94.92125	0.066
9/26/2012	407	407.057 785 800	South Deer	29.27437	-94.91251	0.199
9/26/2012	56	56.015 824 526	South Deer	n/a	n/a	0.199
9/26/2012	168	168.037 831 548	South Deer	n/a	n/a	0.199
10/3/2012	86	86.070 802 085	South Deer	29.26996	-94.91119	0.077
10/3/2012	106	106.021 063 563	South Deer	29.27366	-94.91183	0.077
10/3/2012	383	383.048 591 776	South Deer	29.27070	-94.91006	0.077
10/3/2012	392	392 rt.057 821 114	South Deer	n/a	n/a	0.077
10/3/2012	737	737.070 584 868	South Deer	29.26996	-94.91132	0.077
10/30/2012	180	180.0.37 841 613	South Deer	n/a	n/a	0.085

## Appendix 1. Individual capture location for terrapin during this study.

Date	Notch #	ID	Location	Latitude	Longitude	Tide
10/30/2012	392	392.057 821 114	South Deer	n/a	n/a	0.085
11/6/2012	0	0.057 815 068	South Deer	29.26955	-94.91119	0.065
11/6/2012	1100	1100.048 599 314	South Deer	29.27310	-94.90980	0.065
11/6/2012	56	56.015 824 526	South Deer	29.27036	-94.91260	0.065
11/6/2012	392	392.057 821 114	South Deer	29.27323	-94.90995	0.065
11/28/2012	124.1	124.1.037 830 331	South Deer	29.27415	-94.90995	0.119
11/28/2012	204	204.037 816 838	South Deer	29.27303	-94.90988	0.119
12/5/2012	32	32.048 617 313	North Deer	29.28294	-94.92180	0.106
12/5/2012	666	666.070 604 325	North Deer	29.28285	-94.92468	0.106
12/18/2012	666	666.070 604 325	North Deer	29.28283	-94.92470	-0.087
12/19/2012	124	124.037 830 331	South Deer	n/a	n/a	0.043
12/19/2012	732	732.070 597 840	South Deer	29.27312	-94.91135	0.043
1/7/2013	111	111.057 801 575	South Deer	29.27426	-94.91187	-0.105
1/7/2013	428	428.057 791 098	South Deer	29.27320	-94.90998	-0.105
1/7/2013	770	770.003 031 581	South Deer	29.27320	-94.90998	-0.105
1/17/2013	124.1	124.1.037 830 331	South Deer	29.27107	-94.91187	-0.406
1/24/2013	101	101.021 032 110	South Deer	29.27030	-94.91093	0.015
1/24/2013	408	408.057 786 618	South Deer	29.27304	-94.91151	0.015
1/24/2013	256	256.015 824 526	South Deer	n/a	n/a	0.015
1/24/2013	666	666.070 585 528	South Deer	29.28419	-94.92098	0.015
2/7/2013	167	167.037 819 000	South Deer	29.27335	-94.91357	0.203
2/7/2013	56	56.015 824 526	South Deer	29.27056	-94.91235	0.203
2/7/2013	602	602.070 594 528	South Deer	29.27098	-94.91190	0.203
2/14/2013	124.1	124.1.037 830 331	South Deer	29.27067	-94.91161	-0.011
2/19/2013	540	540.070 808 112	South Deer	29.27261	-94.91286	0.182
3/4/2013	261	261.048 588 824	South Deer	29.27407	-94.91225	0.01
3/4/2013	124	124.1.037 830 331	South Deer	n/a	n/a	0.01
3/4/2013	114	114.021 012 294	South Deer	29.27304	-94.91192	0.01
3/4/2013	180	180.1.019 042 377	South Deer	29.27411	-94.91187	0.01
3/18/2013	102	102.070 581 597	South Deer	29.28881	-94.87361	0.226
3/18/2013	624	624.070 768 272	South Deer	29.27003	-94.91113	0.226
3/18/2013	56	56.015 824 526	South Deer	29.27049	-94.91241	0.226
3/18/2013	180	180.1.019 042 377	South Deer	29.27424	-94.91144	0.226
3/26/2013	152	152.021 045 612	South Deer	29.27046	-94.91074	-0.271
4/15/2013	320	320.048 595 258	South Deer	29.27392	-94.91103	0.228
4/15/2013	168	168.037 831 548	South Deer	29.27407	-94.91023	0.228
4/15/2013	611	611.070 599 548	South Deer	29.27376	-94.91084	0.228
4/15/2013	186	186.037 813 077	South Deer	29.27380	-94.91235	0.228

Date	Notch #	ID	Location	Latitude	Longitude	Tide
4/15/2013	120	120.070 581 597	South Deer	29.27327	-94.91270	0.228
4/15/2013	618	618.070 586 363	South Deer	29.27055	-94.91146	0.228
4/15/2013	295	295.048 611 054	South Deer	29.27424	-94.91159	0.228
4/17/2013	734	734.070 602 598	South Deer	29.27123	-94.91145	0.373
5/16/2013	787	787.070 593 084	Greenslake	29.27449	-94.98545	0.208
5/16/2013	788	788.070 604 045	Greenslake	29.27262	-94.98651	0.208
5/30/2013	792	792.002 891 590	Shell Island	29.45083	-94.92427	0.468
6/5/2013	793	793.003 012 258	Shell Island	29.45091	-94.92442	0.236
6/6/2013	794	794.002 895 843	Shell Island	29.45101	-94.92446	0.297
6/10/2013	795	795.003 027 113	Bolivar	29.52004	-94.54266	0.13
6/13/2013	714	714.070 600 617	Sportsmans	29.25783	-94.90883	0.096
6/21/2013	798	798.003 013 550	Sportsmans	29.25540	-94.91212	0.051
6/24/2013	810	810.003 017 596	Greenslake	29.27535	-94.98567	0.186
6/24/2013	803	803.003 016 524	Greenslake	29.27535	-94.98567	0.186
6/24/2013	804	804.003 019 296	Greenslake	29.27535	-94.98567	0.186
6/26/2013	114	114.021 0123 294	South Deer	29.27286	-94.91145	0.155
6/27/2013	192	192.037 837 038	South Deer	29.27111	-94.91003	0.051
7/16/2013	353	353.048 588 057	Sportsmans	29.25605	-94.91068	0.289
7/16/2013	806	806.003 006 818	Sportsmans	29.25599	-94.91077	0.289
8/1/2013	809	809.003 027 866	South Deer	29.27046	-94.91258	0.033
8/1/2013	705	705.070 784 582	South Deer	29.27046	-94.91258	0.033
8/1/2013	624	624.070 768 272	South Deer	29.27054	-94.91249	0.033
8/1/2013	729	729.070 582 268	South Deer	29.27054	-94.91249	0.033
8/1/2013	811	811.002 889 842	South Deer	29.27040	-94.91225	0.033
8/2/2013	737	737.070 584 868	South Deer	29.27054	-94.91249	0.031
8/7/2013	643	643.070 637 773	Sportsmans	29.25623	-94.91434	-0.045
8/19/2013	468	468.070 812 628	Sportsmans	29.25612	-94.91402	0.199
8/19/2013	821	821.003 015 034	Sportsmans	29.25604	-94.91421	0.199
8/19/2013	820	820.003 012 082	Sportsmans	29.25650	-94.91421	0.199
8/19/2013	353	RT353.048 588 057	Sportsmans	29.25661	-94.91014	0.199
8/19/2013	268	268.037 878 578	Sportsmans	29.25750	-94.90878	0.199
8/19/2013	834	834.003 014 091	Sportsmans	29.25750	-94.98078	0.199
8/28/2013	120-2	120-2.070 581 597	South Deer	29.27362	-94.91260	0.363
8/28/2013	698	698.070 599 337	South Deer	29.27348	-94.91302	0.363
8/28/2013	193	193.037 828 383	South Deer	29.27370	-94.91289	0.363
8/28/2013	247	247.070 579 888	South Deer	29.27232	-94.91381	0.363
9/11/2013	729	729.070 582 268	South Deer	29.27067	-94.91189	0.365
9/11/2013	366	366.048 593 045	South Deer	29.27058	-94.91251	0.365

Date	Notch #	ID	Location	Latitude	Longitude	Tide
9/11/2013	532	532.070 809 115	South Deer	29.27058	-94.91251	0.365
9/18/2013	828	828.003 009 317	North Deer	29.28276	-94.92424	0.407
9/25/2013	25	25.467A373A60	South Deer	29.27395	-94.91089	0.316
9/25/2013	120-2	120.2.070 581 597	South Deer	29.27384	-94.91267	0.316
9/25/2013	574	574.070 583 807	South Deer	29.27182	-94.91243	0.316
9/25/2013	611	611.070 599 548	South Deer	29.27316	-94.91013	0.316
10/2/2013	305	305.048 591 280	North Deer	29.28296	-94.92187	0.281
10/2/2013	346	346.048 589 064	North Deer	29.28317	-94.92177	0.281
10/2/2013	372	372.048 596 020	North Deer	29.28267	-94.92471	0.281
10/2/2013	835	835.003 007 283	North Deer	29.28327	-94.92297	0.281
10/3/2013	353	353.048 588 057	Sportsmans	29.25802	-94.91248	0.311
10/3/2013	468	468.070 812 628	Sportsmans	29.25545	-94.91322	0.311
10/3/2013	486	486.057 782 778	Sportsmans	29.25624	-94.91438	0.311
10/9/2013	838	838.070 796 372	South Deer	29.27131	-94.91293	0.274
10/23/2013	212	212.037 817 100	South Deer	29.27471	-94.91272	0.235
10/23/2013	144	144.037 839 842	South Deer	29.27439	-94.91255	0.235
10/23/2013	320	320.048 595 258	South Deer	29.27471	-94.91272	0.235
10/30/2013	845	845.003 002 334	Sportsmans	29.25597	-94.91093	0.42
10/30/2013	842	842.003 024 793	Sportsmans	29.25554	-94.91335	0.42
10/30/2013	551	551.070 591 048	Sportsmans	29.25580	-94.91027	0.42
10/30/2013	846	846.003 020 012	Sportsmans	29.25458	-94.91001	0.42
11/6/2013	46	46.015 841 329	North Deer	29.28261	-94.92204	0.537
11/6/2013	780	780.070 629 869	North Deer	29.28465	-94.92728	0.537
11/6/2013	847	847.003 004 521	North Deer	29.28322	-94.92256	0.537
11/6/2013	849	849.003 009 281	North Deer	29.28297	-94.92124	0.537
11/14/2013	852	852.003 013 097	Greenslake	29.27097	-94.98927	0.193
11/14/2013	854	854.003 001 078	Greenslake	29.27055	-94.98997	0.193
11/20/2013	855	855.003 015 267	South Deer	29.27320	-94.91187	0.479
11/20/2013	25	25.467A373A60	South Deer	29.27295	-94.91171	0.479
11/20/2013	120.2	120.2.070 581 597	South Deer	29.27389	-94.91261	0.479
11/20/2013	210	210.037 839 592	South Deer	29.27432	-94.91161	0.479
11/20/2013	151	151.021 068 095	South Deer	29.27084	-94.91213	0.479
11/20/2013	459	459.070 806 063	South Deer	29.27236	-94.91245	0.479
11/20/2013	705	705.070 784 582	South Deer	29.27237	-94.91253	0.479

Tide= Highest high tide (m)

Date	Notch #	Location	Capture	R	Image J	L	W	# times checked
3/26/2012	186	South Deer	Random	1	n/a	191	1.3	1
3/26/2012	191	South Deer	Random	1	n/a	183	1.15	1
4/4/2012	167	South Deer	Random	2	1.229	204	1.65	1
4/4/2012	115	South Deer	Random	2	1.297	191	1.4	1
4/23/2012	618	South Deer	Random	2	1.152	195	1.2	1
4/23/2012	698	South Deer	RT	2	1.479	177	0.9	1
4/30/2012	705	South Deer	non Random	3	1.42	177	1.2	1
5/7/2012	707	South Deer	Random	2	1.186	143	0.65	1
5/7/2012	708	South Deer	Random	2	0.96	202	1.45	1
6/6/2012	173	South Deer	Random	1	n/a	183	1.2	1
6/6/2012	618	South Deer	RT	2	1.11	195	1.2	2
8/1/2012	168	South Deer	Random	2	1.24	188	n/a	1
8/1/2012	320	South Deer	Random	2	1.58	182	1.1	1
8/1/2012	695	South Deer	Random	1	n/a	200	1.5	1
8/8/2012	604	South Deer	Random	1	n/a	185	1.15	1
8/8/2012	152	South Deer	Random	1	n/a	196	1.15	1
8/16/2012	727	South Deer	Random	2	1.72	188	1.2	1
8/16/2012	532	South Deer	Random	1	n/a	185	0.95	1
8/16/2012	101	South Deer	Random	2	1.37	190	1.05	1
8/23/2012	729	South Deer	Random	2	1.6	204	1.5	1
8/29/2012	732	South Deer	Random	2	1	214	1.2	1
8/29/2012	56	South Deer	n/a	1	n/a	181	1.15	1
9/5/2012	392	South Deer	Random	2	1.92	197	1.05	1
9/5/2012	760	North Deer	Trap	2	0.94	186	1.25	1
9/26/2012	407	South Deer	n/a	2	1.28	188	1.2	1
9/26/2012	56	South Deer	n/a	1	n/a	181	1.15	1
9/26/2012	168	South Deer	n/a	1	n/a	188	n/a	1
10/3/2012	86	South Deer	n/a	2	1.22	184	1.15	1
10/3/2012	106	South Deer	n/a	1	n/a	199	1.3	1
10/3/2012	383	South Deer	n/a	1	n/a	190	1.25	1
10/3/2012	392	South Deer	n/a	1	n/a	197	1.05	2
10/3/2012	737	South Deer	n/a	1	n/a	190	1.1	1
10/30/2012	180	South Deer	n/a	1	n/a	180	1	1
10/30/2012	392	South Deer	RT	2	1.51	197	1.05	3
Date	Notch #	Location	Capture	R	Image J	L	W	# times checked

Appendix 2:Data collected on individual captured terrapin.

r	1			1	1	1		
11/6/2012	0	South Deer	Random	1	n/a	193	1.25	1
11/6/2012	1100	South Deer	Random	1	n/a	193	1.25	1
11/6/2012	56	South Deer	n/a	2	n/a	181	1.15	2
11/6/2012	392	South Deer	Random	2	1.07	197	1.05	4
11/28/2012	124.1	South Deer	n/a	2	1.72	199	1.35	1
11/28/2012	204	South Deer	n/a	2	0.99	196	1.25	1
12/5/2012	32	North Deer	Random	2	1.39	211	1.68	1
12/5/2012	666	North Deer	Random	2	1.57	183	1.11	1
12/18/2012	666	North Deer	n/a	2	1.61	n/a	n/a	2
12/19/2012	124	South Deer	RT	2	1.86	n/a	n/a	1
12/19/2012	732	South Deer	Random	2	1.53	213	1.69	2
1/7/2013	111	South Deer	Random	2	1.96	203	1.5	1
1/7/2013	428	South Deer	Random	2	1.93	203	1.4	1
1/7/2013	770	South Deer	Random	2	1.05	192	1.35	1
1/17/2013	124.1	South Deer	non Random	2	1.88	n/a	n/a	2
1/24/2013	101	South Deer	Random	2	1.53	190	1.3	2
1/24/2013	408	South Deer	Random	2	1.95	188	1.35	1
1/24/2013	256	South Deer	non Random	1	n/a	n/a	n/a	1
1/24/2013	666	South Deer	non Random	2	1.79	n/a	n/a	3
2/7/2013	167	South Deer	Random	1	n/a	206	n/a	2
2/7/2013	56	South Deer	RT	2	1.24	n/a	n/a	3
2/7/2013	602	South Deer	Random	1	n/a	180	1.11	1
2/14/2013	124.1	South Deer	RT	2	1.84	n/a	n/a	3
2/19/2013	540	South Deer	n/a	2	1.73	171	0.95	1
3/4/2013	261	South Deer	Random	2	1.57	197	1.1	1
3/4/2013	124	South Deer	n/a	n/ a	1.87	n/a		2
3/4/2013	114	South Deer	Random	2	1.63	186	1.2	1
3/4/2013	180	South Deer	Random	2	1.33	191	1.21	2
3/18/2013	102	South Deer	Random	2	1.95	193	1.25	1
3/18/2013	624	South Deer	Random	2	1.35	198	1.35	1
3/18/2013	56	South Deer	RT	2	1.68	181	1.15	4
3/18/2013	180	South Deer	Random	2	1.76	191	1.21	3
3/26/2013	152	South Deer	Random	2	1.73	195	1.15	2
4/15/2013	320	South Deer	Nonrand om	2	1.86	181	1.2	2
Date	Notch #	Location	Capture	R	Image J	L	W	# times checked

4/15/2013	168	South Deer	Random	2	1.66	187	1.25	2
4/15/2013	611	South Deer	n/a	2	1.00	205	1.23	1
4/15/2013	186	South Deer	Random	2	2.01	191	1.71	2
4/15/2013	120	South Deer	RT	1	n/a	193	1.3	1
4/15/2013	618	South Deer	Random	2	1.73	193	1.3	3
4/15/2013	295	South Deer	Random	2	1.75	191	1.3	1
4/17/2013	734	South Deer	Nonrand om	2	1.51	174	0.64	1
5/16/2013	787	Greenslake	Random	2	2.06	203	1.5	1
5/16/2013	788	Greenslake	Random	2	2	205	1.32	1
5/30/2013	792	Shell Island	Pitfall Trap	4	1.59	208	1.4	1
6/5/2013	793	Shell Island	Pitfall Trap	4	1.78	205	1.4	1
6/6/2013	794	Shell Island	Pitfall Trap	4	n/a	201	1.35	1
6/10/2013	795	Bolivar	Random	4	n/a	186	1.3	1
6/13/2013	714	Sportsmans	Random	2	1.43	198	1.2	1
6/21/2013	798	Sportsmans	Random	1	n/a	142	0.45	1
6/24/2013	810	Greenslake	Random	2	2.11	193	1.05	1
6/24/2013	803	Greenslake	Random	1	n/a	158	0.7	1
6/24/2013	804	Greenslake	Random	2	n/a	151	0.55	1
6/26/2013	114	South Deer	Pitfall Trap	4	1.68	186	1.1	2
6/27/2013	192	South Deer	Non Random	1	n/a	194	1.13	1
7/16/2013	353	Sportsmans	Random	2	1.77	195	1.2	1
7/16/2013	806	Sportsmans	Non Random	2	1.07	165	0.73	1
8/1/2013	809	South Deer	Non Random	2	0.901	181	0.88	1
8/1/2013	705	South Deer	Non Random	1	n/a	178	0.93	2
8/1/2013	624	South Deer	Non Random	1	n/a	197	1.29	2
8/1/2013	729	South Deer	Non Random	1	n/a	202	1.31	2
8/1/2013	811	South Deer	Non Random	1	n/a	154	0.7	1
8/2/2013	737	South Deer	Non Random	2	1.48	188	0.95	2
Date	Notch #	Location	Capture	R	Image J	L	W	# times checked

8/7/2013	643	Sportsmans	Random	2	0.75	197	1.05	1
8/19/2013	468	Sportsmans	Random	1	n/a	204	1.59	1
			Non					
8/19/2013	821	Sportsmans	Random	1	n/a	199	1.35	1
8/19/2013	820	Sportsmans	Random	1	n/a	198	1.43	1
8/19/2013	353	Sportsmans	RT	2	1.62	195	1.2	2
8/19/2013	268	Sportsmans	Random	1	n/a	204	1.3	1
8/19/2013	834	Sportsmans	Random	2	1.46	172	0.85	1
8/28/2013	120-2	South Deer	Random	1	n/a	193	1.3	2
8/28/2013	698	South Deer	Random	1	n/a	183	1	2
8/28/2013	193	South Deer	Random	2	1.35	179	0.9	1
8/28/2013	247	South Deer	Random	2	1.52	167	0.74	1
9/11/2013	729	South Deer	RT	1	n/a	202	1.31	3
9/11/2013	366	South Deer	Random	1	n/a	194	1.15	1
9/11/2013	532	South Deer	Random	1	n/a	185	1	2
9/18/2013	828	North Deer	non Random	1	n/a	195	1	1
9/25/2013	25	South Deer	Random	1	n/a	206	1.58	1
9/25/2013	120-2	South Deer	non Random	2	1.68	193	1.3	3
9/25/2013	574	South Deer	Random	1	n/a	194	1.33	1
9/25/2013	611	South Deer	Random	1	n/a	206	1.7	2
10/2/2013	305	North Deer	non Random	2	0.75	179	1.07	1
10/2/2013	346	North Deer	non Random	2	0.76	199	1.5	1
10/2/2013	372	North Deer	Random	1	n/a	201	1.51	1
10/2/2013	835	North Deer	Random	1	n/a	199	1.4	1
10/3/2013	353	Sportsmans	RT	1	n/a	195	1.2	3
10/3/2013	468	Sportsmans	Random	2	0.8	204	1.59	2
10/3/2013	486	Sportsmans	Random	1	n/a	183	1.05	1
10/9/2013	838	South Deer	Random	1	n/a	192	1.2	1
10/23/2013	212	South Deer	Random	2	1.13	184	1.05	1
10/23/2013	144	South Deer	Random	2	1.39	195	0.95	1
10/23/2013	320	South Deer	Random	2	0.99	182	1.05	3
10/30/2013	845	Sportsmans	Random	2	1.15	180	1.05	1
10/30/2013	842	Sportsmans	Random	2	1.04	200	1.4	1

Date	Notch #	Location	Capture	R	Image J	L	W	# times checked
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10/30/2013	551	Sportsmans	Random	2	1.3	181	1.1	1
10/30/2013	846	Sportsmans	Random	2	n/a	189	1.2	1
11/6/2013	46	North Deer	Random	2	1.25	121	1.4	1
11/6/2013	780	North Deer	Random	2	1.39	181	0.85	1
11/6/2013	847	North Deer	Random	2	1.14	184	1.15	1
11/6/2013	849	North Deer	Random	1	n/a	205	1.4	1
11/14/2013	852	Greenslake	Random	1	n/a	197	1.25	1
11/14/2013	854	Greenslake	Random	2	1.43	189	1.25	1
11/20/2013	855	South Deer	Random	2	1.11	170	0.85	1
11/20/2013	25	South Deer	RT	2	1.6	206	1.58	2
11/20/2013	120.2	South Deer	RT	2	1.48	193	1.3	1
11/20/2013	210	South Deer	Random	2	1.52	203	1.3	1
11/20/2013	151	South Deer	Random	2	1.44	211	1.65	1
11/20/2013	459	South Deer	Random	1	n/a	207	1.25	1
11/20/2013	705	South Deer	RT	2	1.35	178	0.93	3

R= Reproductive stage (1= nothing, 2= follicle, 3 = egg and 4 = follicles and eggs)

ImageJ= Image J- Follicle Measurement Length (cm)

L= Length Mid(mm) Carapace

W= Weight (kg)

Capture Method= RT - radio tracking, random - transects, non-random - opportunity capture.

Number times checked=Number of times single terrapin checked with ultrasound over 2012 and 2013 by date of terrapin capture.

Appendix 3: Minitab Read Outs

#### **1. Regression Analysis**

# **1.1.Regression Analysis: Image J- Follicle Measurement versus Carapace length (mm)**

```
The regression equation is

Image J- Follicle Measurement_1 = 0.8294 + 0.003183 Length Mid(mm) Carapace

S = 0.335332 R-Sq = 1.8% R-Sq(adj) = 0.6%

Analysis of Variance

Source DF SS MS F P

Regression 1 0.16808 0.168077 1.49 0.225

Error 80 8.99580 0.112447

Total 81 9.16387
```

#### 1.2. Regression Analysis: Follicle Measurement versus Body condition

The regression equation is Image J- Follicle Measurement\_1 = 1.421 + 0.00089 Body condition S = 0.339872 R-Sq = 0.0% R-Sq(adj) = 0.0% Analysis of Variance Source DF SS MS F P Regression 1 0.00026 0.000261 0.00 0.962Error 79 9.12553 0.115513Total 80 9.12579

#### 1.3. Regression Analysis: Follicle Measurement versus Weight (kg)

```
The regression equation is

Image J- Follicle Measurement_1 = 1.273 + 0.1357 Weight (kg)

S = 0.338323 R-Sq = 0.9% R-Sq(adj) = 0.0%

Analysis of Variance
```

 Source
 DF
 SS
 MS
 F
 P

 Regression
 1
 0.08324
 0.083240
 0.73
 0.396

 Error
 79
 9.04255
 0.114463
 0.114463

 Total
 80
 9.12579
 0.114463

#### 1.4. Regression Analysis: Follicle Measurement versus Log weight

The regression equation is Image J- Follicle Measurement\_1 = 1.406 + 0.4297 Log weight S = 0.337599 R-Sq = 1.3% R-Sq(adj) = 0.1% Analysis of Variance Source DF SS MS F P Regression 1 0.12191 0.121907 1.07 0.304 Error 79 9.00388 0.113973 Total 80 9.12579

#### 1.5. Regression Analysis: Follicle Measurement versus DOY (Day of Year)

The regression equation is Image J- Follicle Measurement\_1 = 1.684 - 0.001175 DOY S = 0.315459 R-Sq = 13.8% R-Sq(adj) = 12.8% Analysis of Variance Source DF SS MS F P Regression 1 1.3897 1.38968 13.96 0.000 Error 87 8.6577 0.09951 Total 88 10.0474

#### 1.6.Regression Analysis: Follicle Measurement versus Tide

The regression equation is Image J- Follicle Measurement\_1 = 1.531 - 0.3967 TIde S = 0.332126 R-Sq = 4.5% R-Sq(adj) = 3.4% Analysis of Variance Source DF SS MS F P 
 Regression
 1
 0.4507
 0.450675
 4.09
 0.046

 Error
 87
 9.5967
 0.110307

 Total
 88
 10.0474

#### 1.7.General Regression Analysis: Follicle Measurement versus DOY, Tide

```
Regression Equation
Image J- Follicle Measurement 1 = 1.69258 - 0.00111126 DOY - 0.154664 TIde +
                                                                            0.000170584 DOY*TIde
 89 cases used, 54 cases contain missing values
Coefficients
                          Coef SE Coef T
Term
                                                                                          Ρ
Constant 1.69258 0.077057 21.9654 0.000
DOY -0.00111 0.000412 -2.6971 0.008
TIde -0.15466 0.376515 -0.4108 0.682
DOY*TIde 0.00017 0.001566 0.1090 0.913
Summary of Model
 S = 0.318500
                                 R-Sq = 14.18%
                                                                           R-Sq(adj) = 11.15\%
PRESS = 9.35530 R-Sq(pred) = 6.89\%
Analysis of Variance

        Source
        DF
        Seq SS
        Adj SS
        Adj MS
        F
        P

        Regression
        3
        1.4248
        1.42484
        0.474946
        4.68194
        0.004479

        DOY
        1
        1.3897
        0.73790
        0.737903
        7.27413
        0.008434

        TIde
        1
        0.0340
        0.01712
        0.017117
        0.16874
        0.682270

        DOY*TIde
        1
        0.0012
        0.00120
        0.001204
        0.01187
        0.913496

        Error
        85
        8.6226
        8.62258
        0.101442
        1.54500
        0.083364

Lack-of-Fit 46 5.5674 5.56743 0.121031 1.54500 0.083364

Pure Error 39 3.0551 3.05515 0.078337

Total 88 10.0474
```

#### 2. One-Way ANOVA

#### 2.1.One-way ANOVA: Follicle Measurement versus Location

Method

Null hypothesisAll means are equalAlternative hypothesisAt least one mean is differentSignificance level $\alpha = 0.05$ Rows unused55

Equal variances were assumed for the analysis.

### **Tukey Pairwise Comparisons**

Grouping Info	rmat	ion Usin	g the Tukey Method and 95% Confidence
Location	Ν	Mean	Grouping
Greenslake	4	1.900	A
Shell Island	2	1.6850	AB
South Deer	63	1.4864	AB
Sportsmans	10	1.239	В
North Deer	9	1.200	В
Means that do	not	share a	letter are significantly different.

#### 2.2.One-way ANOVA: Follicle Measurement versus Month

\* NOTE \* Cannot draw the interval plot for the Tukey procedure. Interval plots for comparisons are illegible with more than 45 intervals.

Method

```
Null hypothesisAll means are equalAlternative hypothesisAt least one mean is differentSignificance level\alpha = 0.05Rows unused54
```

```
Equal variances were assumed for the analysis.
Factor Information
Factor Levels Values
Month 12 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12
Analysis of Variance
Source DF Adj SS Adj MS F-Value P-Value
Month 11 2.997 0.27249 2.98 0.002
Error 77 7.050 0.09156
Total 88 10.047
Model Summary
        S
            R-sq R-sq(adj) R-sq(pred)
0.302587 29.83% 19.81% 0.00%
Means
Month N Mean StDev
                                    95% CI
         7 1.727 0.334 (1.499, 1.955)
1
        3 1.603 0.319 (1.255, 1.951)
9 1.6522 0.2112 (1.4514, 1.8531)
2
3
      12 1.5214 0.3120 (1.3475, 1.6954)
4
5
        5 1.559 0.486 (1.290, 1.829)
        5 1.622 0.376 (1.353, 1.891)
6
7
        2 1.420 0.495 (0.994, 1.846)
8
       13 1.3532 0.2999 (1.1860, 1.5203)

      4
      1.455
      0.433
      (1.154, 1.756)

      11
      1.0945
      0.2556
      (0.9129, 1.2762)

      13
      1.3454
      0.2200
      (1.1783, 1.5125)

      5
      1.5920
      0.1712
      (1.3225, 1.8615)

9
10
11
12
```

## Pooled StDev = 0.302587

#### **Tukey Pairwise Comparisons**

Grouping Information Using the Tukey Method and 95% Confidence

Month	Ν	Mean	Grouping
1	7	1.727	A
3	9	1.6522	A
6	5	1.622	АB
2	3	1.603	АB
12	5	1.5920	АB
5	5	1.559	АB
4	12	1.5214	A
9	4	1.455	АB

7	2	1.420	АB				
8	13	1.3532	ΑB				
11	13	1.3454	ΑB				
10	11	1.0945	В				
Means	that	do not	share a	letter	are	significantly	different.

#### **3.Follicle Presence**

#### 3.1.Binary Logistic Regression: Binary versus DOY, Weight (kg)

Link Function: Logit Response Information Variable Value Count 1 86 (Event) 0 46 Binary Total 132  $\star$  NOTE  $\star$  132 cases were used \* NOTE \* 11 cases contained missing values Logistic Regression Table Odds 95% CI SE Coef Z P Ratio Lower Upper SE Coet \_\_\_\_\_\_ 2.92304 -0.11 0.909 \_\_\_\_\_\_ 0.33 0.743 Predictor Coef -0.335584 Constant 0.0041473 0.0126555 0.33 0.743 0.98 1.00 1.03 DOY 2.34988 0.69 0.492 Weight (kg) 1.61423 5.02 0.05 502.66 DOY\*Weight (kg) -0.0071523 0.0100552 -0.71 0.477 0.99 0.97 1.01 Log-Likelihood = -82.573Test that all slopes are zero: G = 5.532, DF = 3, P-Value = 0.137 Goodness-of-Fit Tests Chi-Square DF P 118.485 118 0.470 152.329 118 0.018 Method Chi-Square Pearson Deviance 8 0.131 12.492 Hosmer-Lemeshow Table of Observed and Expected Frequencies: (See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic) Group 7 Value 1 2 3 4 5 6 8 9 10 Total 1 Obs 7 7 7 8 8 11 11 12 86 11 4 Exp 7.0 7.3 7.6 7.9 8.2 8.4 8.6 9.4 10.0 11.5 0 Obs 3 6 6 6 9 5 5 2 2 2 46 3.0 Exp 7.0 5.7 5.4 5.1 4.8 4.6 4.4 3.6 2.5

95%

CI

Total 14 13 13 13 13 13 13 13 13 14 132 Measures of Association: (Between the Response Variable and Predicted Probabilities) Pairs Number Percent Summary Measures Concordant 2358 59.6 Somers' D 0.20 39.2Goodman-Kruskal Gamma0.211.2Kendall's Tau-a0.09 Discordant 1551 Ties 47 3956 100.0 Total

#### **3.2.Binary Logistic Regression: Binary versus DOY, Carapace length (mm)**

Link Function: Logit Response Information Variable Value Count Binary 1 87 (Event) 0 48 135 Total \* NOTE \* 135 cases were used \* NOTE \* 8 cases contained missing values Logistic Regression Table Odds Coef SE Coef Z Predictor P Ratio Lower Constant -4.88524 7.56175 -0.65 0.518 0.0294833 0.0326987 0.90 0.367 1.03 0.97 DOY Length Mid(mm) Carapace 0.0334041 0.0397324 0.84 0.401 1.03 0.96 DOY\*Length Mid(mm) Carapace -0.0001758 0.0001708 -1.03 0.303 1.00 1.00 Predictor Upper Constant 1.10 DOY Length Mid(mm) Carapace 1.12 DOY\*Length Mid(mm) Carapace 1.00 Log-Likelihood = -85.131Test that all slopes are zero: G = 5.460, DF = 3, P-Value = 0.141 Goodness-of-Fit Tests Chi-Square DF Method Ρ 129.609 122 0.302 164.716 122 0.006 Pearson Deviance 8 0.086 Hosmer-Lemeshow 13.829

Table of Observed and Expected Frequencies:

(See Hosmer-Lemeshow Test for the Pearson Chi-Square Statistic)

					Gr	oup					
Value 1	1	2	3	4	5	6	7	8	9	10	Total
										13 11.3	87
0											
Obs	3	8	7	5	7	8	4	2	3	1	48
Exp	6.9	6.3	5.4	5.5	4.8	4.9	4.2	4.1	3.3	2.7	
Total	13	14	13	14	13	14	13	14	13	14	135

Measures of Association: (Between the Response Variable and Predicted Probabilities)

Pairs	Number	Percent	Summary Measures	
Concordant	2644	63.3	Somers' D	0.27
Discordant	1502	36.0	Goodman-Kruskal Gamma	0.28
Ties	30	0.7	Kendall's Tau-a	0.13
Total	4176	100.0		