

Dispersal of Newly-Emerged Diamond-backed Terrapin (*Malaclemys terrapin*) Hatchlings

at Jamaica Bay, NY

THESIS

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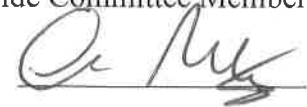


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ABSTRACT

Overwintering behavior of diamond-backed terrapins (*Malaclemys terrapin*) is enigmatic; they may be the only aquatic turtles in which the hatchlings routinely spend their first winter on land outside the nest. However, many aspects of this behavior are poorly documented. Previous observations describing long-term usage of wrack lines as an overwintering refugium are anecdotal. Three hundred forty one neonate *M. terrapin* hatchlings were implanted with RFID tags and released at their nest sites in Jamaica Bay, New York, and tracked until spring emergence. Eighty one hatchlings over 3 field seasons were successfully tracked from their nests to their overwintering refugia and over the winter. Hatchling *M. terrapin* used the wrack line only as short term cover, instead they moved to upland terrestrial refugia. Micro- and macro-habitat conditions around successful overwintering refugia were compared to unsuccessful refugia. Hatchlings were tracked to determine date of emergence from refugia. Average % ground cover, canopy height, % forb cover, average forb height, and average tree height were higher for successful refugia than for refugia where hatchlings left before spring emergence. Average ground cover, canopy height, forb height, grass height, % bush, % tree, and tree height within a 5m radius of successful overwintering sites were higher than at unsuccessful sites. Hatchling refugia depths were variable, up to 10cm deep, with some vertical movement seasonally. Hatchlings emerged from overwintering refugia between 17 March and 7 July. In Jamaica Bay hatchling *M. terrapin* do not utilize the wrack line, which is periodically flooded, as cover for overwintering refugia. The advantage of overwintering outside the natal nest may be to avoid desiccation from drier nesting sites. These findings add to a little known aspect of the *M. terrapin* life history.

INTRODUCTION

The diamond-backed terrapin, *Malaclemys terrapin* (Schoepff, 1793) is found in brackish waters along the United States Atlantic and Gulf coasts from Cape Cod, Massachusetts to Corpus Christi, Texas. Females come ashore to nest between April and July (Ernst and Lovich 2009). In New York the *M. terrapin* nesting period is from 3 June to 23 July with an average clutch size of 12 eggs (Feinberg 2000, Feinberg and Burke 2003). Nests are laid above the high water line in areas of sparse vegetation, loose soil and high sunlight incidence (Roosenburg 1994). Shrubland, dune, and mixed grassland are preferred nesting habitat (Feinberg and Burke 2003). Nests are susceptible to depredation by predators such as raccoons (*Procyon lotor*, Burger 1977, Rulison 2009), red foxes (*Vulpes fulva*), crows (*Corvus sp.*) and laughing gulls (*Larus atricilla*, Burger 1977). Root predation from nearby forbs and grasses can also cause nest failure (Feinberg and Burke 2003). The incubation period is prolonged by decreasing temperatures, nest depth, as well as for nests with a later nesting date (Roosenburg 1996, Burger 1976). In New Jersey the average incubation period for eggs laid in June and July were 74.5 and 86.0 days, respectively (Burger 1976). *M. terrapin* hatchlings emerge from nests in both fall and spring (Baker et al. 2006, Gibbons and Nelson 1978, Lazell and Auger 1981, Ultsch 2006, Gibbons 2013).

As for nearly all turtle species, little is known about *M. terrapin* hatchling ecology. Hatchlings are predated by raccoons (Muldoon and Burke 2012, Rulison 2009), and laughing gulls (Burger 1976), Norway rats (*Rattus norvegicus*, Draud et. al 2004, Muldoon and Burke 2012) and Black-crowned night herons (*Nycticorax nycticorax*, Burger 1976). *M. terrapin* hatchlings have been found in intertidal high marsh

vegetation,, tidal mud flats, dense vegetation, and tidal wrack lines (Draud et al. 2004, Lovich et al. 1991, Pitler 1985). The wrack line can be described as accumulated debris deposited by the highest tide. While adult *M. terrapin* hibernate from mid to late November to April-May in shallow marsh creeks and in the mud in the intertidal zone (Yearicks et al. 1981), hatchling *M. terrapin* apparently do not spend their first winter in the water or intertidal zone. Instead, hatchlings either overwinter within the natal nest (Gibbons and Nelson 1978, Lazell and Auger 1981, Baker et al. 2006, Ultsch 2006, Gibbons 2013) or emerge from the nest and overwinter terrestrially elsewhere (Draud et al. 2004, Muldoon and Burke 2012). Adaptations to this overwinter on land include freeze tolerance (Baker et al 2006) and anoxia intolerance (Dinkelacker et al 2005). Some *M. terrapin* hatchlings overwinter in the nest, others emerge from the nest in the fall and seek alternate refugia (Ultsch 2006, Baker et al. 2006, Graham 2009). Those that overwinter on land, especially at high latitudes, are subjected to subzero temperatures and require a physiological mechanism for surviving freezing temperatures.

Freeze tolerance limits for *M. terrapin* have not been determined, but in laboratory trials hatchlings survived freezing to -3.0°C for 3 days and up to 12 days at -2.5°C (Baker et al. 2006). Overwintering hatchlings in natural nests experienced less extreme temperatures for shorter durations than the laboratory trials (Baker et al. 2006).

Hatchlings of many other Emydidae turtles are similarly freeze tolerant and anoxia intolerant, but all others are known to overwinter in the nest (Ultsch 2006). However, the strategy of overwintering terrestrially outside the nest (TON) is only known among Emydidae in *M. terrapin* and Eastern box turtles (*Terrapene carolina*) (Burke and

Capitano 2011). It is not known what habitat is utilized by hatchling *M. terrapin* overwintering terrestrially, or what the adaptive significance of this behavior might be.

Where do terrapin hatchlings spend their first winter?

Physiological constraints may dictate aspects of *M. terrapin* hatchling habitat choice. Terrapins live in estuarine systems, where salinities can vary widely; they can reach as high as 28 ppt during the summer months (Davenport and Macedo 1990). Dunson and Mazzotti (1989) showed that *M. terrapin* hatchlings without access to fresh drinking water did not grow in sea water concentrations with salinity above 21ppt, but would grow in 9 ppt salinity. Calichio (unpub. data) found that *M. terrapin* hatchlings cannot even survive in salinities of 9ppt water without access to fresh water, so it is very unlikely they can spend much time in ocean water. Even if terrapin hatchlings spend some time in the wrack line presumably they must at least occasionally find fresh or brackish drinking water.

A number of reports indicate that some *M. terrapin* hatchlings spend their first winter on land. Capturing 324 individuals over the spring and fall of two years, Muldoon and Burke (2012) found that at least some terrapin hatchlings in Jamaica Bay overwinter somewhere well above the tide line, perhaps in the upland vegetation. Nine laboratory-raised *M. terrapin* hatchlings released into the water in an estuary quickly sought refuge in the flotsam of the high tide wrack line (Lovich et. al. 1991). This indicates that *M. terrapin* juveniles do not readily use open water habitats, as do the adults, but use near shore habitats instead. Similarly, Pitler (1985) found that 12 juvenile *M. terrapin* over a 3 year period used beach surface debris as well as matted *Spartina* grass, dense low lying vegetation, and a rock, all on well-drained ground about 91 meters

from the water edge at low tide. Burger's (1976) study of *M. terrapin* hatchling behavior during and after emergence from their nests found that hatchlings always moved to nearby vegetation. Draud et al. (2004) found predated hatchling *M. terrapin* carcasses 0.5 – 35m from the mean high tide line during the period of spring emergence from terrestrial overwintering hibernacula, and of 24 individual hatchlings tracked via radio telemetry, 16 were killed by rats. All of these suggest that terrapin hatchlings used wrack lines and near-shore Salt meadow cordgrass (*Spartina patens*) dominated habitats. No other aquatic Emydidae is known to depend on terrestrial habitats as hatchlings.

M. terrapin hatchling diet analysis also indicates they use near shore habitats. Hatchlings forage on prey species from both aquatic and terrestrial habitats, inhabiting the transitional intertidal zone (King 2007). Terrestrial feeding by hatchling *M. terrapin* may reflect their inability to develop under osmotic stress in a highly saline environment (Kinneary 2008).

The purpose of this study was to explore TON in *M. terrapin* hatchlings by 1) identifying the conditions under which post-emergent hatchlings overwinter, 2) determining whether hatchlings overwintered in the high tide wrack line, and 3) describing TON microhabitats and determining what features influence refugia choice.

Determining which habitats *M. terrapin* hatchlings utilize after nest emergence will illuminate a poorly known aspect of its early life history for the species. Though aquatic throughout its adult life, insight into its early requirements, when it is vulnerable to predators and habitat loss, has conservation/management implications. Preservation of upland habitat may have the dual purpose of protecting nesting sites and hatchling refugia sites.

METHODS

Study Area

Rulers Bar Hassock (48° 36' 58.68" N 73° 50' 07.63" W) is a 458 ha man-made island in the Jamaica Bay Wildlife Refuge, part of Gateway National Recreation Area, and administered by the National Park Service (NPS). At the time of this study, a 1,730 meter long gravel trail encircled West Pond, an 18.21 hectare mildly saline impoundment. The island is surrounded tidal beachfront backed by dunes interspersed with *Spartina* marsh. The west beach generally has a substantial wrack line that abuts the dune edge and is fully inundated during peak high tides. Vegetation coverage from the dune top to the edge of West Pond varies from sparse ground cover to a full canopy. The NPS routinely cuts large sections of vegetation down to ground level up to 20m from the trail edge. Ground cover varies from none to 100% and is comprised of woody debris, forb material, leaf litter, rock, or combination thereof.

Rulers Bar Hassock is maritime coastal habitat (Rulison and Burke 2012); upland habitat (110 ha) categorized under the National Vegetation classification system is dominated by Northern Tall Maritime Shrubland, Successional Maritime Forest and Early Successional Woodland Forest (Edinger et al. 2008). Separating the North Atlantic Upper Beach (6 ha) from the upland vegetative zones are Eastern Tidal Salt Shrub North, Atlantic Coast Backdune Grassland, Northern Bayberry Dune Shrubland, Overwash Dune Grasslands, and Northern Beachgrass Dune. Ninety eight hectares are estuarine and categorized as North Atlantic Low Salt Marsh, North Atlantic High Salt Marsh,

Woolgrass Marsh, Mixed Forb Marsh, and Reed-grass Tidal Marsh. Estuarine Intertidal Mud comprises an additional 23 ha of land space (Edinger et al. 2008).

Jamaica Bay tides mix ocean water and freshwater twice daily. Average salinity towards the center of the bay can vary from 23 to 27 parts per thousand (NYCDEP 2007), but near shore salinities can be even higher. At high tide salinities were found to be between 28-30 ppt and low tide salinities were found to be 31-32 ppt (Callichio, pers com.).

Rulers Bar Hassock is reported to be the largest *M. terrapin* nesting site in New York. In 1999 an estimated 2053 nests were laid at the island (Feinberg and Burke 2003), and 95% of terrapin nests found in the western portion of Jamaica Bay are laid on Rulers Bar Hassock (Ner and Burke 2005).

Pit Tag Insertion

I marked newly emerged *M. terrapin* hatchlings captured at their nest sites. The majority of hatchlings were captured at nest sites located as part of a larger ongoing, long-term study of *M. terrapin* ecology. Female *M. terrapin* were observed nesting in June and July, after completion of oviposition and nest covering the nests were protected from predation by wire mesh enclosures. During the fall hatchling emergence period (August –October) nests were checked at least once, sometimes multiple times a day. On 19 December 2009, 20 December 2009, and 19 March of 2011 ~15 meters of wrack line was manually searched for *M. terrapin* hatchlings.

In 19 September through 10 October 2009 I inserted 9×2.12 mm (0.067g) Passive Integrated Transponder (PIT) tags (Biomark, TX148511B) into 60 *M. terrapin* newly emerged from nests (Duncan 2013). I similarly tagged 36 hatchlings from 13

August- 2 October 2010, and 251 hatchlings from 15 August - 27 September 2011. Tag insertion generally followed Rowe and Kelly (2005) except that tags were not inserted via needle injection. Instead, prior to tag insertion I cleaned the skin in the left inguinal region with 99% ethanol and made a 2–3 mm long but shallow incision into the body wall with a recurved scalpel blade to avoid excessive cutting of musculature and internal organs. I inserted a single sanitized (soaked for 10 minutes in 99% ethanol) PIT via the incision into the intraperitoneal region with sanitized tweezers. Once the PIT was no longer visible, I once again cleaned the wound with ethanol and covered it with a quick-drying liquid bandage (CVS, Liquid Bandage™). Hatchlings were observed a minimum of 20 min and up to 24 hr post-insertion; release sites were within 1 m of capture sites or nests (Duncan 2013).

Hatchling Searches

After hatchling releases, area searches were conducted with a Biomark FS2001 Reader and portable antenna. The reader was worn in a chest harness and the attached antenna was mounted on a handle and waved a few centimeters over or in direct contact with the ground much like a metal detector. Searches were generally concentrated on potential cover such as wrack lines, under dense vegetation and areas of leafy and woody debris. Failure to locate particular individuals was followed by a more methodical grid search centered on the release point (Duncan 2013).

Refugia Locations

Whenever possible hatchlings were observed from a distance of >10m upon release to observe their general direction of movement, and where possible hatchlings were followed to a refugia. If a hatchling moved and was located at another site the

original site was considered a temporary refugium. At each refugium I noted the distance from release site as well as distance from the West Pond and Jamaica Bay shoreline, in intervals of >5m, 5-10m, 10-25m, 25-50m, and >50m.

I also described the habitat type of each overwintering refugium or temporary refugium site (beach, wrack line, dune face, dune top, open sand patch, edge vegetation, interior vegetation, trail, marsh grass, or brush pile). I estimated percent ground cover and described the major ground cover (forb material, woody debris, rock) at hatchling locations. Percent grass, forb, bush, and mature tree and heights in meters were determined for each as well as canopy height, were also visually estimated for circular area with a 25cm² radius immediately surrounding each refugium, and again for the circular areas with a 5m radius immediately surrounding each refugium. Similar site descriptions were recorded each time a hatchling moved to a new location, and distance and direction from previous location were recorded.

Refugia were checked periodically through the winter until the following July, even when no PIT tag signal was detected, in case hatchlings had buried too deeply for detection, and then moved back within the range of the reader. Hatchlings were considered to have left their refugia in the spring if after March 1 their signal could not be located after multiple attempts over two weeks in the area where it had been previously located.

Excavation of Refugia

After 1 July, refugia with PIT signals were considered to have dead hatchlings or that hatchlings had shed the tags. These were excavated carefully by hand to recover tags or hatchling remains.

Three refugia were chosen randomly in 2012 to measure the depth of hatchlings (Table 4); these were excavated, one time until each hatchling was partially exposed, depth was quickly measured, and soil replaced. One hatchling was checked twice (23 March 2012 and 6 April 2012) when the ground surface was apparently disturbed within that time period, possibly by a predator after the first excavation.

Statistical Analysis

Logistic regression was used to compare the habitat characteristics (% ground cover, % grass, grass height, % bush, bush height, % tree, tree height, % forb cover, forb height, and canopy height) within one meter of sites where hatchlings successfully overwintered (n = 58) with sites where they buried themselves but did not successfully overwinter (apparently died)(n = 10). I then conducted univariate regression analysis with each of the ten variables.

Logistic regression was used to compare the same ten habitat characteristics measured over the area within five meters of sites where hatchlings successfully overwintered (n = 54) with sites where they buried themselves but did not successfully overwinter (apparently died)(n = 8).

Logistic regression was used to compare the same 10 habitat characteristics measured over the area within one meter of sites where hatchlings overwintered (n = 68) with sites they occupied only temporarily (n = 45) before moving on to overwintering sites.

Logistic regression was also used to compare the same ten habitat characteristics measured over the area within five meters of sites where hatchlings overwintered (n = 62)

with sites they occupied only temporarily ($n = 38$) before moving on to overwintering sites.

RESULTS

Hatchling Locating

Three hundred forty one hatchlings were captured when they emerged from their nests; these were implanted with PIT tags and released at their nests. Six additional hatchlings were found during a wrack line search on 21 September 2009, implanted with PIT tags and returned to the wrack line. Of 347 hatchlings implanted with PIT tags and released, 205 (59%) were never located again. Of the 142 found at least once after tagging, 81 were found in overwintering refugia until spring when they likely emerged. Seventy seven were found in only 1 location where they overwintered, 3 were located in two separate refugia (a temporary, later abandoned and a successful overwintering refugium) each, and 1 that settled in an overwintering refugium after abandoning two temporary sites. Hatchlings ($n=81$) were found in successful overwintering refugia on average 5.91 m (std dev =7.04) from the original nest site. The range of distance from the nest site was 0.1m - 47m (Figure 1). Nine hatchlings marked at their nests moved towards the water from their nests and hid in the wrack line.

Hatchlings in the Wrack Line

Of the 15 hatchlings found in the wrack line, 6 were originally found in the wrack line during a visual search for hatchlings and returned there after PIT tags were implanted. The remaining 9 were located after release from nests on dunes 5m or less from the wrack line. None of the hatchlings tagged and returned to the wrack line or found to have moved to the wrack line from their natal nest were detected in the wrack

line past 11 October (Table 1). The maximum amount of time hatchlings were known to use the wrack line was 19 days. Eight hatchlings found in the wrack line were only found there once, the same day as tagging and release. One hatchling was found twice in the wrack line, having moved to a new location the day after it was tagged and released. One hatchling left its temporary refugium in the wrack line for a different temporary refugium, 8 days after it was initially tagged and released.

Spring Emergence

In 2010 hatchlings left their refugia 25 April to 2 July (n=6, avg 19 May 2012, std dev 34.4 days), in 2011 the analogous dates were 9 April to 13 May (n=8, avg = 25 Apr 2011, std dev = 15.06 days), and in 2012 it was 17 March to 12 May (n=67, avg = 14 Apr 2012, std dev 11.02 days) (Table 2)(Fig 2).

Depth of Refugia

Twelve pit tags that had been implanted in hatchlings were recovered buried in overwintering refugia or on the ground surface. These tags were either expelled from hatchlings or were from dead hatchlings (Table 3). Three of the tags recovered in 2010 were found with hatchling remains, but the cause of mortality could not be determined. One tag and remains were found within 5cm of the surface while a second was 5-10cm deep. A third tag found with remains was 8-10cm deep. All were recovered on the same date, 22 July 2010. The remaining 9 tags without hatchling remains were found at varying depths from on the ground surface to 5cm below the surface.

Hatchlings excavated in their refugia (N= 4) were found up to 6cm below the surface (Table 4). One hatchling was checked twice; the first time it was 6 cm and the

second time 3-4 cm deep. After the initial exhumation, a disturbance of the exact site up to a few centimeters below the surface indicated possible evidence of predation, despite a signal from the PIT it had moved upward 2-3 cm and horizontally 3-4 cm, wedging itself within a root system, apparently in response to disturbance.

Refugia Site Analysis

Logistic regression used to compare the habitat characteristics (% ground cover, % grass, grass height, % bush, bush height, % tree, tree height, % forb cover, forb height, and canopy height) within one meter of sites where hatchlings successfully overwintered (n = 58) with sites where they buried themselves but did not successfully overwinter (apparently died)(n = 10), was statistically significant ($X^2(10) = 19.3$, $P=0.037$), explained 45.8% (Nagelkerke R^2) of the variance in success and correctly classified 88% of the cases. Univariate regression analysis with each of the ten variables showed none was significant (all $P > 0.072$), thus suggesting that a complex of characters and not a single character may explain the observed patterns.

The logistic regression was to compare the same ten habitat characteristics measured over the area within five meters of sites where hatchlings successfully overwintered (n = 54) with sites where they buried themselves but did not successfully overwinter (apparently died)(n = 8), was not statistically significant ($X^2(10) = 6.8$, $P=0.744$). The model explained 19.4% (Nagelkerke R^2) of the variance in success and correctly classified 87% of the cases.

Logistic regression used to compare the same 10 habitat characteristics measured over the area within one meter of sites where hatchlings overwintered (n = 68) with sites they occupied only temporarily (n = 45) before moving on to overwintering sites, was

statistically significant ($X^2(10) = 35.6$, $P < 0.001$). The model explained 37.5% (Nagelkerke R^2) of the variance in success and correctly classified 77.3% of the cases. Two variables (% ground cover and % forb cover) were significant contributors to the model ($P = 0.035$ and 0.013 , respectively); all other variables had $P = 0.077$. Temporary sites had lower % ground cover ($x=76.9$, $s.d. = 35.0$) and lower % forb cover (7.8 , $s.d. = 12.8$) than overwintering sites ($x = 97.1$, $s.d. = 11.7$ and 18.4 , $s.d. = 27.9$, respectively).

Logistic regression used to compare the same ten habitat characteristics measured over the area within five meters of sites where hatchlings overwintered ($n = 62$) with sites they occupied only temporarily ($n = 38$) before moving on to overwintering sites, was statistically significant ($X^2(10) = 22.5$, $P = 0.013$). The model explained 27.7% (Nagelkerke R^2) of the variance in success and correctly classified 66.2% of the cases. Two variables (% ground cover and % grass cover) were significant contributors to the model ($P = 0.022$ and 0.011 , respectively); all other variables had $P = 0.067$. Temporary sites had % ground cover ($x=60.4$, $s.d. = 26.2$) and % grass cover (17.8 , $s.d. = 18.0$) levels similar to overwintering sites ($x = 63.1$, $s.d. = 18.5$ and 18.2 , $s.d. = 19.2$, respectively).

Ground cover at successful refugia sites averaged 96% ($n=71$ $std\ dev = 9.57$) compared to an average of 62% ($n=54$, $std\ dev = 19.09$) for ground cover within a 5m radius of abandoned refugia sites.

DISCUSSION

The ability to tolerate whole body freezing by hatchling *M. terrapin* is known in 3 other Emydidae: the painted turtle (*Chrysemys picta*), Blanding's turtle (*Emys blandingii*), and ornate box turtle (*Terrapene ornata*) (Baker et al. 2003). *M. terrapin*

hatchlings also have the ability to supercool, resisting freezing to ~ 15 °C below the equilibrium freezing/melting point (Baker et al. 2006). Northern map turtles (*Graptemys geographica*) the sister taxon of *Malaclemys*, may lack freeze tolerance but shares the ability to supercool (Baker et al. 2003). These two traits should be tested in more *Graptemys*, to explore evolution of overwintering strategies in this clade. As with *M. terrapin* overwintering within natal nests, Baker et al. (2003) showed that *G. geographica* overwinter in nests (approximately 12cm in deep). Thus *G. geographica* nest depths are shallower than observed nest depths of *M. terrapin* in Jamaica Bay (15.05 cm, Scholz 2006) and New Jersey (14.98cm, Montevecchi and Burger 1975). However, *G. geographica* hatchlings have not been observed to emerge from nests terrestrial for refugia (TON) as reported here for *M. terrapin* (Baker et al. 2003). Investigating overwinter nest temperatures in *G. geographica* and *M. terrapin* at similar latitudes should indicate whether these close relatives experience similar nest conditions.

Movement From the Nest

M. terrapin hatchling overwintering refugia were 4-5m from the natal nest site (Figure 1). Of the 142 located refugia, 72 were less than 5m from the natal nest site. Of the 81 located successful overwintering refugia 48 were less than 5m from the natal nest site. One possible explanation for this result could be that as hatchlings traveled farther from the nest, I was more likely to lose them, despite intensive search protocols. Another possibility is that hatchlings generally do not disperse far from their nests. Leaving the relative safety of the natal nest site even for a short distance increases the risk of predation. Hatchlings may need to travel long distances to find a suitable overwintering refugium. Some natal nests may meet the conditions of suitable refugia but hatchlings

may relocate due to competition within the nest for resources such as moisture.

Alternatively, hatchlings may depart the natal nest to avoid ice nucleating agents if freezing increases the chance of mortality despite being freeze tolerant. Ice nucleating agents can inhibit hatchling turtle's ability to supercool by 8-10 °C (Costanzo et al. 2000).

Use of the Wrack Line

It does not appear that wrack line is important to *M. terrapin* hatchlings at Jamaica Bay as overwintering refugia. No hatchlings from nests more than 5m from the wrack line were found in the wrack line; suggesting that hatchlings may only use the wrack line when it is the closest cover. Burger (1976) found that emerging hatchlings preferred to move toward vegetation and if an incline was present preferred movement downslope; wrack lines are down slope from most Jamaica Bay terrapin nests. Jamaica Bay wrack lines vary in thickness seasonally when present but could be utilized in some instances as temporary vegetative cover. However, on numerous occasions I observed hatchlings climbing up the dune face from nest site and away from a substantial wrack line contradicting Burger's earlier observations.

Of the 15 hatchlings that took refuge within the wrack line, 7 could not be found more than 24 hours later. They may have been depredated; raccoon tracks and evidence of raccoon foraging were abundant around the wrack line after hatchlings were found. There is some evidence that raccoons prey on hatchlings either after nest emergence (Rulison 2009) or within the nest after emergence has begun (Burger 1977).

Spring Emergence

Muldoon and Burke (2012) reported two separate pulses of hatchling sightings annually over 14 years; the first April-July and peaking May-June, the second August - October. The phenology of the spring pulse is consistent with dates I detected hatchling emergence from overwintering refugia 2010 – 2012 (Fig. 2). The possibility that the spring pulse of hatchling sightings could have recently emerged from overwintering refugia instead of overwintering in their nests in this population is consistent with the finding that only 8 of 122 observed JB *M. terrapin* nests had overwintering hatchlings (Ultsch 2006). Therefore hatchlings observed in the spring are more likely to have terrestrially overwintered outside the nest (TON) as opposed to overwintering inside the nest (OWN).

Mortality

There are two studies available examining PIT tag retention in Emydidae turtles. Runyon and Meylan (2005) found that of 28 pit-tagged *Trachemys scripta* and 8 *Pseudemys floridana* and 3 *Pseudomys nelsoni*, only 1 tag was lost in the first month, and that was probably due to poor tag placement. Post-implant recaptures showed tags still in place as long as 50 months after initial insertion. Six recaptured *Trachemys scripta* out of seven PIT-tagged turtles 5 were known to have retained their tags at 16 months and 3 were known to have retained tags a minimum of 24 months (Bulhmann and Tuberville 1998). Only one study has tested tag retention in hatchling turtles; of 8 hatchling *C. picta* with tags inserted in peritoneal cavities, two tags were partially expelled and had to be reinserted within the first ten days (Rowe and Kelly 2005).

Some of the tags I recovered without hatchling remains (Table 3) may have been expelled tags as Rowe and Kelly (2005) found. However, mortality associated with the

three tags I found with hatchling remains may have been due to predation or another source of mortality, possibly associated with PIT tags and insertion. Despite care to sanitize instruments and tags, insertions were conducted under field conditions and infections may have occurred.

Refugia Depth and Seasonal Movement Within

M. terrapin hatchling refugia depth may vary over the winter, and hatchlings may burrow deeper into hibernacula beyond the range of the reader. According to the manufacturer (Biomark), the antenna can scan through wood, soil, and water, although the signal is influenced by metals and other electronics and the read range of the antenna at highest power is optimal when a tag is oriented vertically. The effective read range of the portable antenna with a 2.4 m cable is 9.4 cm with the tag at a parallel orientation and 26.7 cm when the tag is oriented perpendicularly (Anonymous 2011). During the 2011-2012 field season 2 hatchlings were located on October 9 and December 27 respectively. Despite frequent attempts to detect their PIT again at the same locations, signals for both hatchlings were not detected again until April 14, which is well within the range of known emergence dates from refugia in 2012 (Table 2). The hatchlings were alive all winter, and appear to have been buried beyond the scanner detection range, and then moved back towards the surface in the spring. Hatchling refugia may extend to depths up to and perhaps greater than 10cm. This is further supported by hatchling remains recovered up to depths of 10 cm.

Refugia Site Selection

Hatchling *M. terrapin* may select refugia based on a suite of above ground environmental factors; clearly some sort of vegetative cover is important at the refugia

site. Successful overwintering refugia have a higher frequency of grass, forb, bush, and tree cover than unsuccessful overwintering refugia (Fig 4, Fig 5). The most important criteria for a successful overwintering refugia site may be the percentage of ground cover. The importance of ground cover is emphasized by comparing the percent ground cover for the composition types of leaf litter, forb material, woody debris, wrack line, rock, and trash, between successful overwintering refugia and abandoned overwintering refugia (Fig.3). Successful overwintering refugia have a much greater percentage of ground cover than non-successful overwintering refugia. Movement between refugia may reflect searching for an aspect of substrate cover necessary for successful overwintering.

Soil moisture may be an important factor for *M. terrapin* hatchling successful overwintering refugia, and relevant to the TON vs. TIN (Terrestrially overwintering inside the nest) strategies generally. Sunny locations are preferred as shown by *M. terrapin* in Maryland (Roosenburg 1996). Hatchling *M. terrapin* may seek alternate refugia with higher moisture content to avoid desiccation. Muldoon and Burke (2012) suggest that desiccation may have reduced body size of hatchlings overwintering on land.

Further Research

Desiccation is underappreciated as a possible source of mortality in turtles, yet it may be especially important to terrestrially overwintering hatchlings because of their high surface area/volume ratios. Investigation into the below-ground conditions experienced by hatchling *M. terrapin* in their refugia is warranted. For example, a comparison of soil moisture content in nest sites versus refugia through a winter may indicate why hatchlings leave nests for refugia, because they may seek refugia with higher moisture content if desiccation is a serious mortality risk to overwintering

hatchlings. This would be consistent with possible terrestrial overwintering Blanding's turtle hatchlings (*Emys blandingii*) that choose overwintering sites on the edge of wetlands in damp soil (Link and Gillette 2009). Some ground cover types may also provide better barriers to subsurface moisture loss. The microclimates refugia with different ground cover types should also be explored.

Hatchling *M. terrapin* may utilize wrack lines as overwintering refugia more in other parts of their range, such as locations where the wrack line is larger and more consistent. *M. terrapin* hatchling behavior should be studied elsewhere in their range to identify overwintering strategies or use of the wrack line in more southern climes where *M. terrapin* may not brumate.

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Table 1. Hatchling locations within the wrack line after nest emergence.

<u>Tag Number</u>	Date Tagged	Found/Release Site	Date Released	Date of First Location in Wrack	Distance from Release Point	Date of Second Location	Distance of Movement	Date of Last Known Locating in Wrack
40516	9/18/2010	dune nest	9/18/2010	9/18/2010	1.5m			9/18/2010
40512	9/21/2009	West beach wrack line	9/21/2009	9/21/2009	none			9/21/2009
40538	9/21/2009	West beach wrack line	9/21/2009	9/21/2009	none			9/21/2009
40550	9/21/2009	West beach wrack line	9/21/2009	9/21/2009	5 m			9/21/2009
40565	9/21/2009	West beach wrack line	9/21/2009	9/21/2009	none			9/21/2009
40519	9/21/2009	West beach wrack line	9/21/2009	9/21/2009	none			10/11/2009
40552	9/21/2009	West beach wrack line	9/21/2009	9/21/2009	none	9/22/2009	10m (in wrack)	10/11/2009
40563	9/22/2009	dune nest	9/22/2009	9/23/2009	3m			9/30/2009
40537	9/22/2009	dune nest	9/22/2009	9/25/2009	6m			10/11/2009
40566	9/23/2009	dune nest	9/25/2009	9/25/2009	?			9/25/2009
40560	9/25/2009	dune nest	9/26/2009	9/26/2009	3.5m			9/26/2009

40575	9/25/2009	dune nest	9/26/2009	9/26/2009	4.5m	10/4/2009	2.5m	observed 10/4/2009
40511	9/25/2009	dune nest	9/26/2009	9/30/2009	3.5m			10/10/2009
40528	9/25/2010	dune nest	9/25/2010	9/25/2010	3m			9/26/2010
79336	9/4/2011	dune nest	9/4/2011	9/4/2011	4.5 m	9/5/11		9/4/2011

Table 2. Date of last location of hatchlings in overwintering hibernacula.

Tag number	Date of first location	Distance from natal nest	Date of last location
Year 2011-2012			
78278	2/4/2012	8.2m	4/11/2012
78333	9/11/2011	0.2m	4/6/2012
78347	3/17/2012	5.8m	4/28/2012
78449	4/6/2012	3.3m	4/28/2012
78451	2/4/2012	0.2m	4/14/2012
78503	3/17/2012	3.6m	4/11/2012
78553	3/17/2012	7.3m	4/14/2012
78607	10/22/2011	3.2m	4/6/2012
78616	10/22/2011	3.5m	4/14/2012
78617	10/22/2011	3.5m	4/19/2012
78671	3/17/2012	2.3m	4/28/2012
78778	2/4/2012	0.9m	4/14/12
78779	9/24/2011	3.2m	3/23/12
78913	3/23/2012	9.6m	3/23/12
78915	12/26/2011	9.1m	4/14/12
78960	3/17/2012	7.0m	4/6/12
78995	4/6/2012	4.1m	4/11/12
79049	12/26/2011	2.75m	4/19/12
79105	10/22/2011	14.9m	4/19/12
79179	3/17/2012	7.4m	4/14/2012
79217	3/17/2012	0.9m	4/14/2012
79296	9/9/2011	2.0 m	4/28/2012
79322	3/17/2012	2.6m	3/17/2012
79336	9/4/2011	11m	4/28/2012
79346	2/18/2012	13m	4/14/2012
79408	4/14/2012	2m	4/19/2012
79434	2/4/2012	0.6m	4/14/2012
79440	2/4/2012	0.9m	5/5/2012
79495	9/24/2011	3.4m	5/5/2012
79553	9/24/2011	1.8m	4/6/2012
79592	12/26/2011	7.35m	4/14/2012
79648	4/19/2012	2.0m	4/28/2012
79649	12/26/2011	11.4m	4/14/2012
79829	3/23/2012	6.9m	3/23/2012
79915	2/18/2012	3.2m	4/28/2012
80004	12/26/2011	7.4m	4/11/2012
80022	3/23/2012	11.5m	4/11/2012

80026	12/10/2011	8.4m	4/6/2012
80151	4/6/2012	2.5m	4/19/2012
80229	9/24/2011	0.2m	4/14/2012
80268	3/17/2012	4.4m	4/14/2012
80284	4/6/2012	6.5m	4/11/2012
80385	9/27/2011	6.1m	5/5/2012
80427	3/17/2012	3.9m	4/14/2012
80676	9/17/2011	2.0m	5/12/2012
80709	12/26/2011	11m	4/14/2012
80810	9/11/2011	2.3m	4/14/2012
80815	9/27/2011		4/6/2012
80865	9/24/2011	8.8 m	4/28/2012
80880	4/11/2012	5.4m	5/5/2012
80969	9/24/2011	2.2 m	4/11/2012
81045	2/4/2012	0.4m	4/11/2012
81142	2/4/2012	12.4m	4/11/2012
81175	4/11/2012	4.2n	5/5/2012
81306	3/23/2012	5.0m	4/14/2012
81380	3/17/2012	2.0m	4/14/2012
81403	9/4/2011	0.25 m	4/28/2012
82601	2/4/2012	11.5m	4/14/2012
83266	8/19/2011	34 m	4/14/2012
83802	12/10/2011	2.6m	4/19/2012
84176	12/10/2011	15.4m	4/19/2012
84405	9/27/2011	4.5m	4/19/2012
84951	9/11/2011	4.1m	4/14/2012
85141	10/22/2011	2.2m	4/11/2012
85186	4/6/2012	10m	4/28/2012
85839	2/18/2012	4m	4/6/2012
86218	4/6/2012	47m	4/6/2012
Year 2010-2011			
40406	9/26/2010	2m	4/9/2011
40507	3/19/2011	11m	5/13/2011
40526			4/23/2011
40551	10/3/2010	3m	4/30/2011
40579	9/26/2010	1m	5/7/2011
40597	10/3/2010	3m	5/13/2011
40736	10/2/2010		4/9/2011
40750	10/26/2010	2m	4/9/2011
Year 2009-2010			
40453	9/25/2009	4m	5/8/2010
40553	9/23/2009	1m	7/2/2010

40573	9/25/2009	1m	7/2/2010
40584	9/30/2009	3m	4/25/2010
40592	10/11/2009	18m	5/1/2010
40598	9/30/2009	1m	4/17/2010

Table 3. Depth of recovered PIT tags

Tag #	Nest #	Date Implanted	Date Recovered	Depth	Hatchling Remains
40531	A	9/25/2009	7/17/2010	0-3cm	N
40541	B	9/25/2009	7/22/2010	3-5cm	N
40555	A	9/25/2009	7/22/2010	3-5cm	Y
40583	A	9/25/2009	7/22/2010	3-10cm	Y
40579	C	9/23/2009	7/22/2010	8-10cm	Y
86189	D	9/17/2011	5/19/2012	4cm	N
85854	D	9/17/2011	5/19/2012	3-4cm	N
85178	D	9/17/2011	5/19/2012	4cm	N
84441	E	9/4/2011	5/12/2012	unk	N
83529	D	9/17/2011	5/19/2012	2cm	N
82401	D	9/17/2011	5/19/2012	Surface	N
79057	F	8/29/2011	5/19/2012	2-3cm	N

Table 4. Depth of hatchlings in refugia excavated before spring emergence

Tag #	Date of First Location	Date excavated	Depth
80676	9/17/2011	5/12/2012	under leaf litter
78347	3/17/2012	3/23/2012	6cm
78347	3/17/2012	4/6/2012	~3-4cm
79217	3/17/2012	3/23/2012	3-4cm

Figure 1. Distance (m) of neonate hatchling refugia from natal nest site.

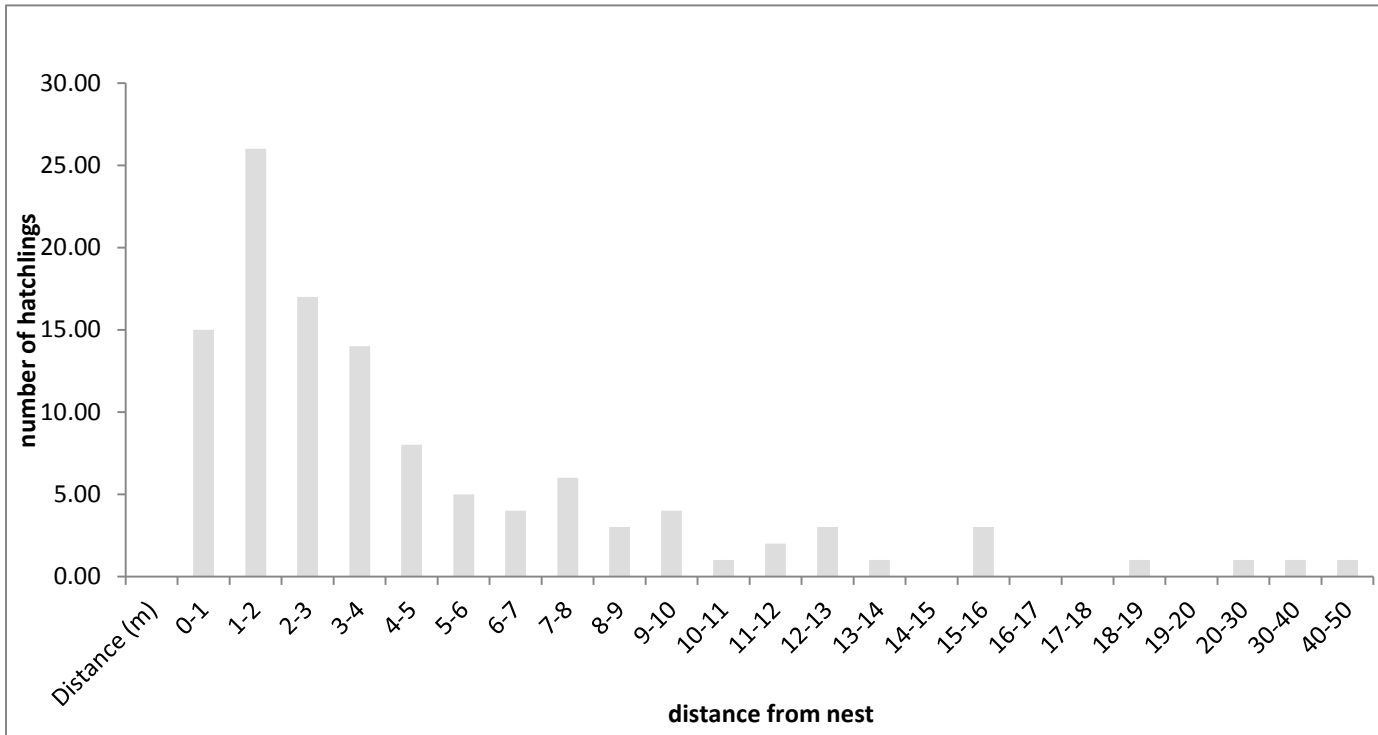
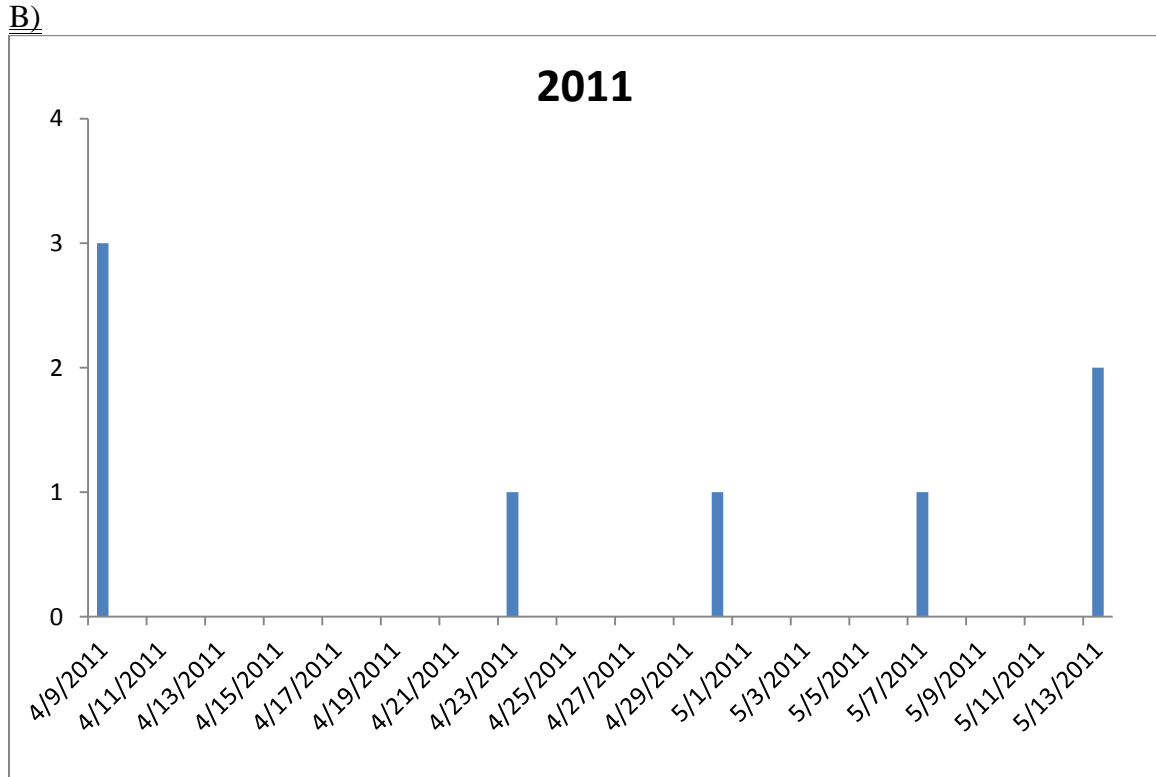
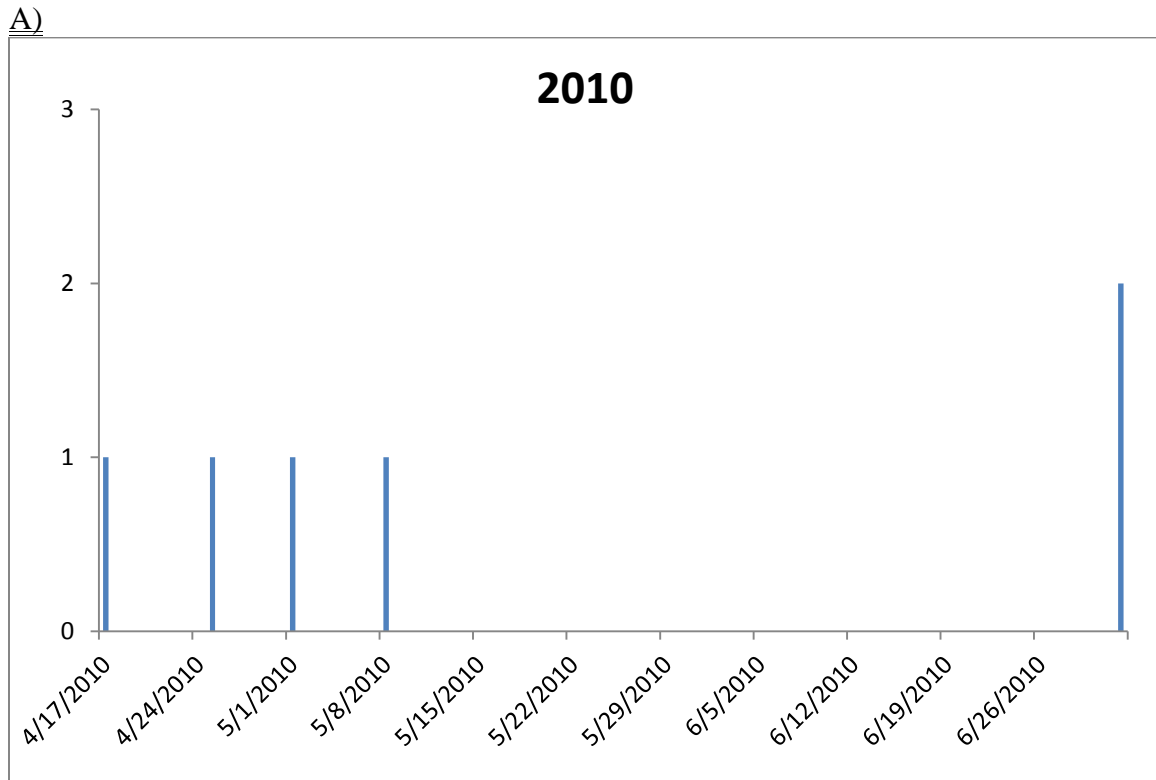
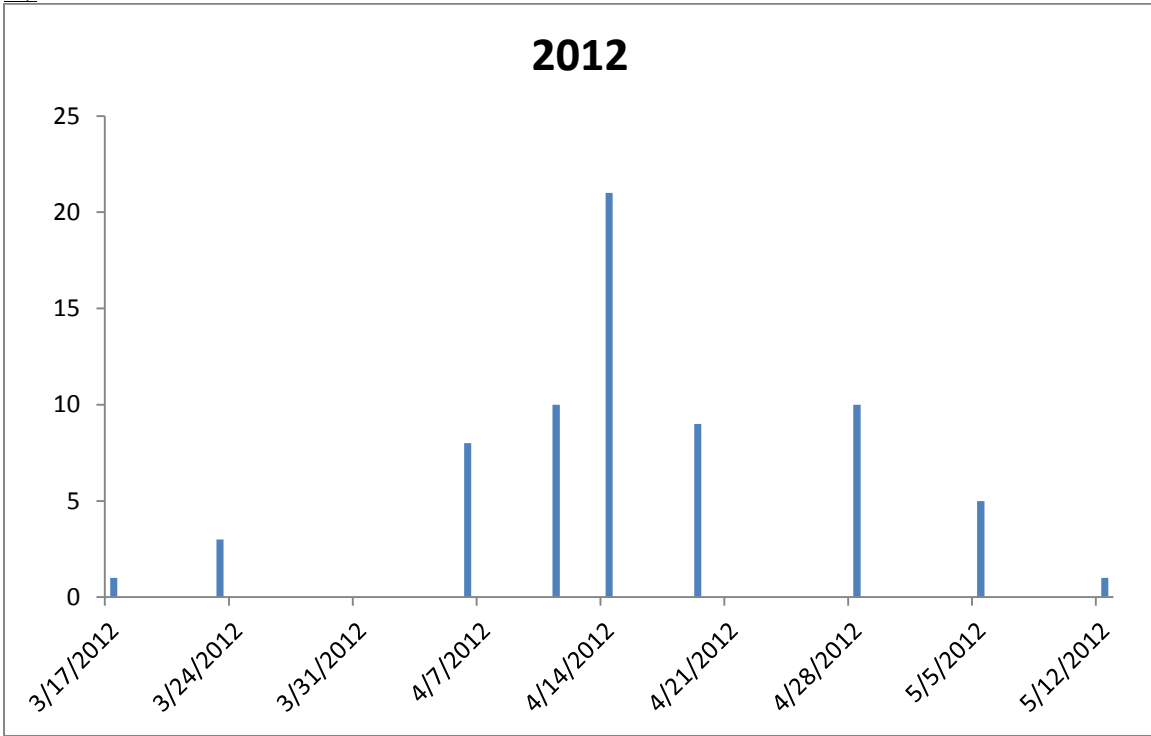


Figure 2. Dates of hatchling emergence from successful overwintering refugia.



C)



D)

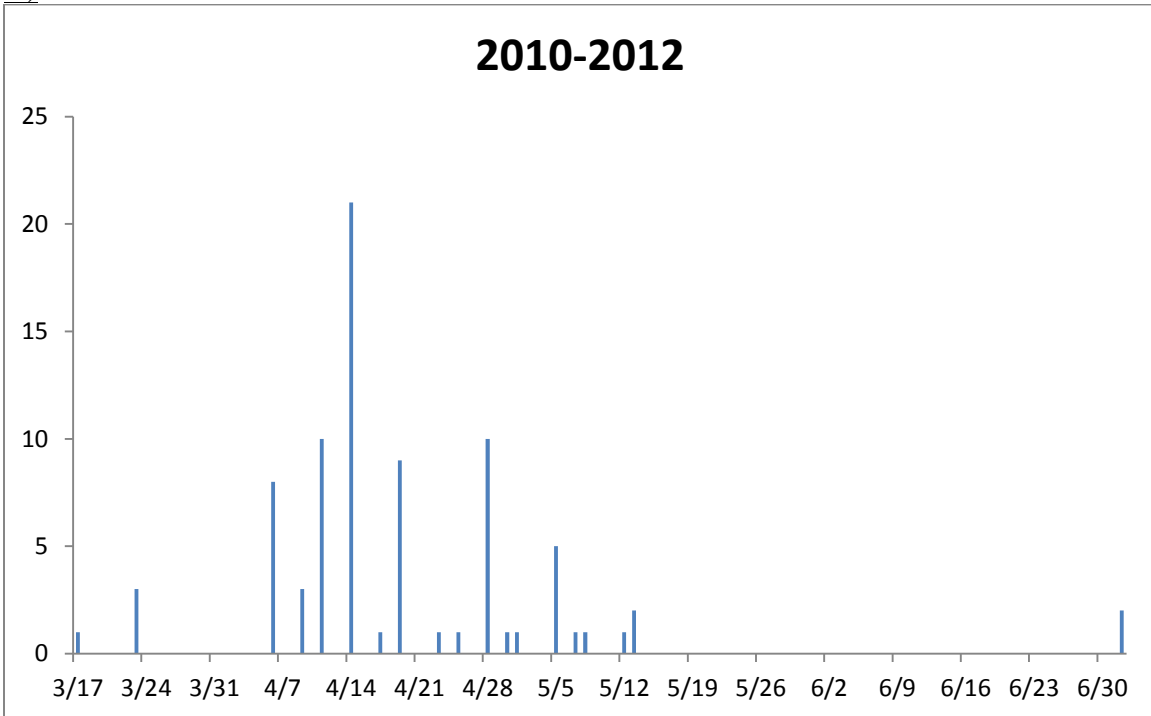


Figure 3. Percent Ground Cover Composition for Successful Overwintering Refugia vs. Non Successful Overwintering Refugia.

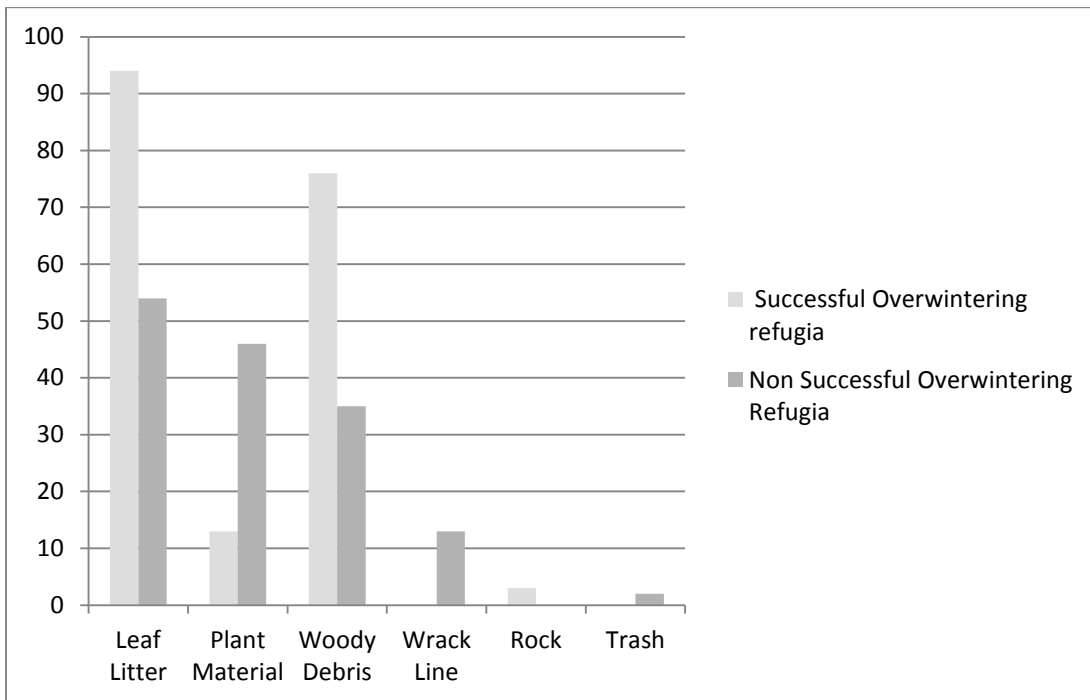


Figure 4. Percent ground cover, grass cover, and forb cover, within one meter of overwintering sites and temporary sites, displayed as means and standard deviations.

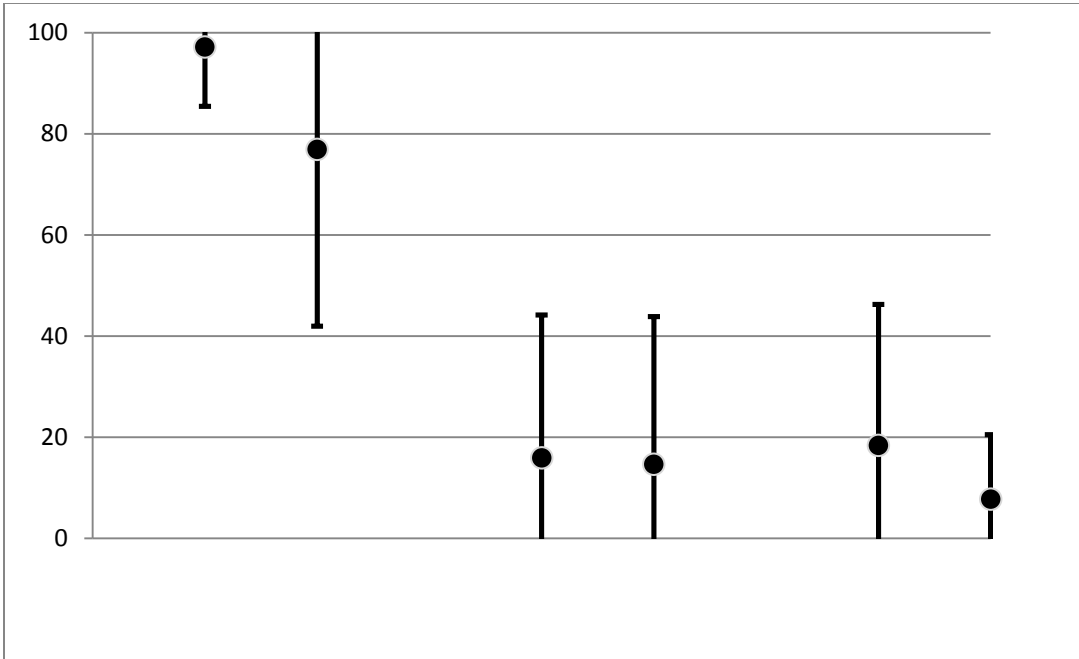


Figure 5. Percent ground cover, grass cover, and forb cover, within five meters of overwintering sites and temporary sites, displayed as means and standard deviations.

