ANNUAL REPORT

South Carolina State Wildlife Grant SC-T-F13AF01208

South Carolina Department of Natural Resources

October 1, 2014 – September 30, 2015

Project Title: An Evaluation of Culturing Carolina Diamondback Terrapins for Responsible Stock Enhancement

Objective (Year 1): **Evaluate the feasibility of culturing diamondback terrapins by altering diet to determine optimal culture growth.**

Accomplishments: The effect of three different diets (2 commercially produced turtle foods, 1 natural diet) on terrapin growth were evaluated from October 2014 through April 2015. Diets were either ZooMed (4 mm pellets), Mazuri (4 mm pellets) or a natural diet of fileted and skinned Atlantic croaker (*Micropogonias undulatus*) and spot (*Leiostomus xanthurus*). One hundred and eighty laboratory-hatched terrapins (90 females, 90 males) were selected for the feed study in early October based on size (no significant difference in hatchling size). Sixty individuals were randomly assigned to each treatment with equal female/male representation. Individuals are identified by a unique numeric identifier written on the carapace with oil-based markers in three different colors based on diet assignment. Prior to the start of the study, all terrapins were acclimated to a pellet or natural diet by offering only one food choice per feeding; in this way we ensured that all animals were feeding at the beginning of the study, no matter which feed treatment they were assigned to. Terrapins were housed in seven "raceway" tanks at a constant temperature of 25^{o}C with low salinity recirculating well water (5-10 ppt), plastic plants for shelter, and basking platforms. Each week, individuals were randomly reassigned to one of seven tanks in order to randomize any potential tank effects (physical or behavioral interactions) across feed treatments. Terrapins were fed four times per week by removing them from their randomized tanks and placing them in diet-specific feeding bins (15 terrapins/bin). Enough food was offered so that terrapins could feed to satiation over the period of 90 – 120 minutes before returning them to their randomized tanks. All terrapins were measured (carapace length, carapace width, plastron length, mass) at the beginning of the experiment and monthly to track growth.

Overall, from October to April, we observed significant differences in growth between the 3 diets (ANOVA: $F_{3,1417}=717.994$, $R^2=0.603$, $p<0.0001$; Figure 1). Monthly growth measurements began to show statistical differences starting in January 2015 ($F_{2,177}=4.309$, $R^2=0.047$, $p=0.015$). At that time, turtles fed the two pelleted diets (Zoo Med and Mazuri) began to show higher growth than the turtles fed a diet of fish (Figure 2). By the end of our study in April 2015, turtles fed on both of the pelleted diets were significantly larger than those fed the fish diet (Figure 2). These findings lead to two major conclusions:

1) Diamondback terrapins fed just fish showed stunted growth and a softer shell, likely because they require a more varied diet. We know from previous diet studies that diamondback terrapins not only eat fish, but also small estuarine organisms, including fiddler crabs, periwinkles, mud snails and other benthic invertebrates (Tucker et al. 1995). For the purposes of headstarting terrapins, our study emphasizes that if a "natural" diet is used, it should consist of a combination of several organisms more comparable to the diet of a wild turtle.

2) Commercial pelleted diets are acceptable diets to use for headstarting diamondback terrapins. There was no statistical difference between the two pelleted diets however, on average, the Mazuri diet resulted in larger individuals (Figure 2). The pelleted diets we used resulted in above average growth trajectories, compared to wild terrapins, and was a more efficient use of project money and time when compared to using a more "natural" diet.

Significant deviations: None.

Objective (Year 2): **Capture gravid female diamondback terrapins and collect eggs using two techniques: in-water turtle capture and harvest on nesting beach.**

Accomplishments: During the 2015 mating/nesting season (May 1 through July 9), 155 diamondback terrapins (86 male/69 female) were captured using trammel nets. Of the 69 females captured, 60 were gravid. All terrapins were captured in the Charleston Harbor system, which includes sites in the Ashley River, Wando River and lower Charleston Harbor. Female terrapins were captured both during routine fishery-independent survey by the SCDNR Inshore Fisheries Section (targeting recreationally important species of fishes) as well as during terrapin-targeted sampling trips. We also obtained twenty females (eleven gravid) on land from Plum Island, Charleston, SC, as they made their overland movement in search of suitable nesting habitat. In total, 71 gravid female terrapins were collected during this season and clutches were obtained from all.

Terrapins were brought back to the laboratory to be sexed, measured (head width, carapace length, carapace width, plastron length, shell depth), weighed, and marked on three unique marginal scutes using an alphabetical code (A through X beginning on the right marginal scute and moving clockwise). All females were scanned by ultrasound to detect the presence of follicles or eggs; those that had either follicles or shelled eggs were held in one of two outdoor tank systems (flow-through ambient Charleston Harbor water) and all remaining females and males were released at the site of capture. The females retained for egg harvest were fed a natural diet (fish, snails, mussels and crabs) while in captivity and were rescanned every 2 – 5 days to assess follicle/egg development. Females that had only follicles or a combination of eggs and follicles were held in a completely aquatic tank. Once only shelled eggs were visible in the ultrasound images and could be easily felt by palpation, females were placed in a tank system consisting of an aquatic tank connected by a bridge to a tank filled with construction sand. The sand filled tank was meant to offer a "natural" habitat for females to construct nests. Five females made nests in the sand tank. All other females were induced to lay eggs using oxytocin. Females were brought into the laboratory and injected with 2 units of oxytocin/100 g of body weight (Tucker et al. 2007). They were then placed in a plastic bin of water individually so when eggs were laid they were not accidentally crushed by the female terrapin. Females were injected every 2 hours, up to 3 times/day until all eggs were collected (as determined through ultrasound). The time from injection to egg-laying varied from 30 minutes to multiple days. Once egg-laying began, the time until all eggs were released ranged from 10 minutes to multiple days. Following egg collection, we collected a tail tip from each female terrapin for genetic analysis before releasing them at their original site of capture.

Significant deviations: Trammel netting was used as the primary method of collection, either deployed parallel to the marsh or set and retrieved in a manner similar to a seine net. The idea of using silt fence/pitfall trap arrays was abandoned due to better catch efficiency from trammel net captures and several failed attempts due to extremely high tides, winds and significant storm events.

Objective (Year 2): **Incubate eggs at varying temperature ranges in order to produce 50% male hatchlings and 50% female hatchlings. Assess differential growth and hatching success between terrapins caught by different methods (in-water vs. on nesting beach; Table 1).**

Accomplishments: Each clutch of eggs collected from gravid female diamondback terrapins was measured (length and width in mm) and weighed (grams), buried halfway in Hatchrite nesting substrate in a plastic container and placed in one of two incubators. One incubator was set at 27^{o}C to produce male hatchlings and the other was set to incubate at 31^{o}C to produce female hatchlings. Eggs began hatching on June 24, 2015 and the last hatchling emerged on August 27, 2015. Incubation time varied from 42 to 62 days with an average of 47 days for females and 59 days for males. Overall hatching success for all eggs was 90 % resulting in 394 individuals, which consisted of 214 females and 180 males. All hatchlings were measured once their yolk was fully absorbed and their plastron healed (carapace length, plastron length, carapace width, depth and mass). Carapace length averaged 31.8 mm (Range: 21.1-35.1 mm), plastron length averaged 28.2 mm (Range: 21.3-31.7 mm) and mass averaged 8.22 grams (Range: 5.14-10.92 grams). There was no statistical difference between male and female size at hatching.

Significant deviations: None.

Objective (Year 2): **Continue to track "headstarted" terrapins (2014 year class) through the winter to identify winter hibernacula habitat of juvenile terrapins**

Accomplishments: Six diamondback terrapins were affixed with radio transmitters and released in May 2015. Immediately after the release, a combination of extremely high tides and storms prevented us from tracking individuals. Tracking efforts since then have failed to locate any of the six terrapins, probably because they dispersed further than usual during the extreme tides. We are continuing to actively search for them using radio telemetry.

Significant deviations: Due to the difficulty in finding the original six individuals affixed with transmitters, and the extremely tides associated with abnormally high rainfalls during 2015, we have not yet released any more individuals fitted with transmitters. Instead, we will attempt to answer the question of preferred overwintering habitat of yearlings by including them in our Year 3 objective below.

Objective (Year 3): **Assess the overwintering habitat preference of age 0 terrapins.**

Accomplishments: In October 2015, a pen with a 500 ft perimeter fence encompassing 5 high marsh microhabitats was constructed in Grice Cove, Charleston, SC. One hundred and eighty-six terrapin hatchlings from the 2015 year class and 17 yearlings from the 2014 year class were each equipped with a PIT tag. The individuals selected for this experiment were hatchlings acquired from females captured in Grice Cove. This was precautionary, in the event that an individual escaped from the pen, it would be genetically similar to the wild population. Individuals were released in the high marsh enclosure in mid-November, allowing them time to select a suitable microhabitat within the enclosure before burying for winter. After a couple weeks of acclimation to the penned area, we will be able to scan the pen with a reader and locate individuals throughout the winter. In addition, temperature loggers placed in different micro-habitats will be used to assess spatial and temporal differences in temperature. Upon emergence in the spring, individuals will be relocated, measured and weighed so that their growth can be compared to

headstarted individuals kept in the laboratory. Each hatchling will be marked and released in Grice Cove the following spring.

Significant deviations: We have added 17 yearlings from the 2014 year class to the overwintering pen study to compare and contrast microhabitat use and survival with hatchlings.

Literature Cited:

Tucker, A.D., N.N. Fitzsimmons and J.W. Gibbons. 1995. Resources Partitioning by the Estuarine Turtle *Malaclemy terrapin*: Trophic, Spatial, and Temporal Foraging Constraints. Herpetologica 51(2):167-181.

Tucker, J.K., D.L. Thomas and J. Rose. 2007. Oxytocin Dosage in Turtles. Chelonian Conservation and Biology 6(2):321-324.

Estimated Federal Cost: $71,516.53 (amount spent through 9/30/2015).

Recommendations: Continue the grant.

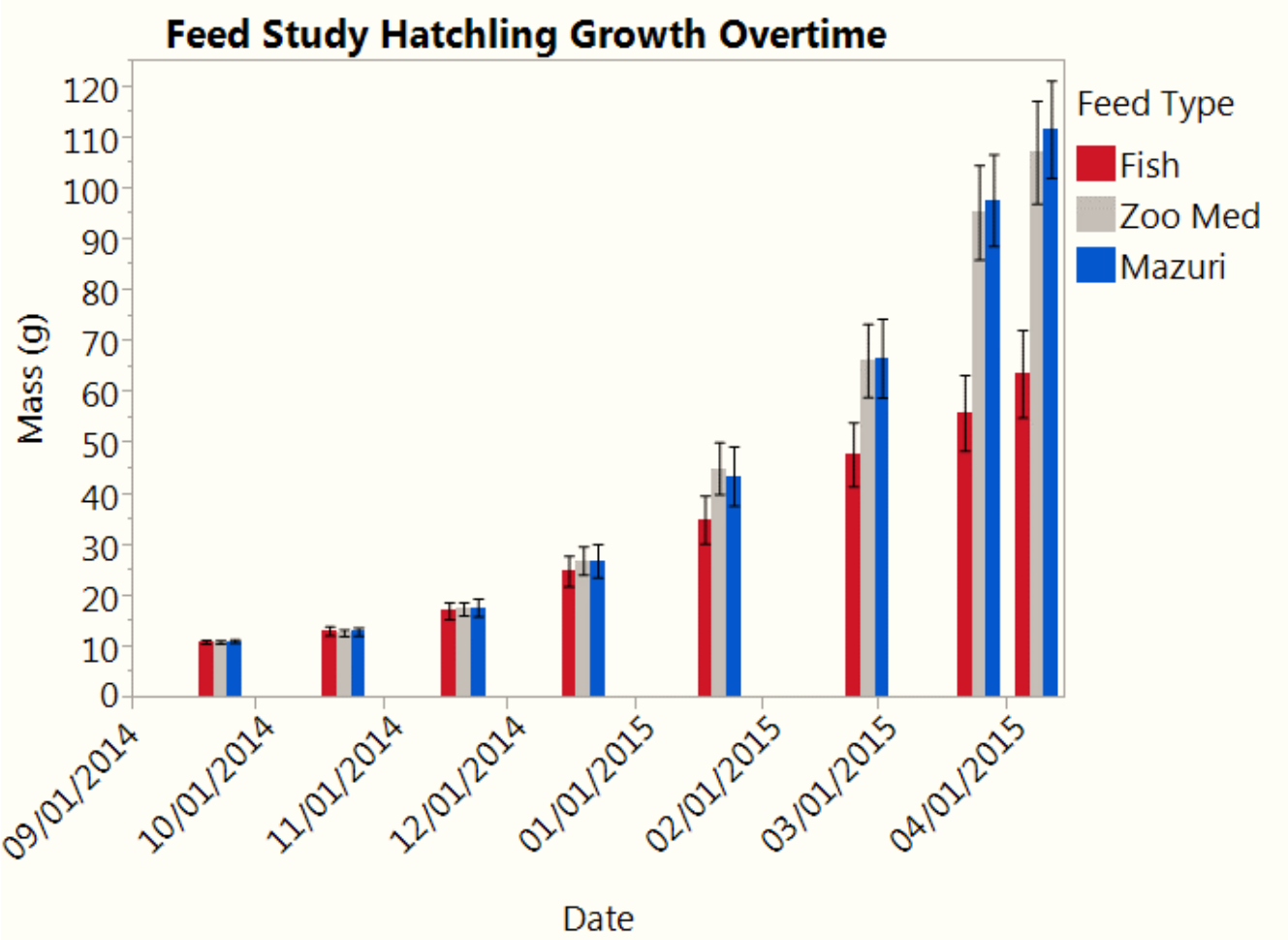

Figure 1. Diamondback terrapin hatchling monthly growth when offered an exclusive diet of one of three diets (Zoo med commercial pellet, Mazuri commercial pellet or fish filets).

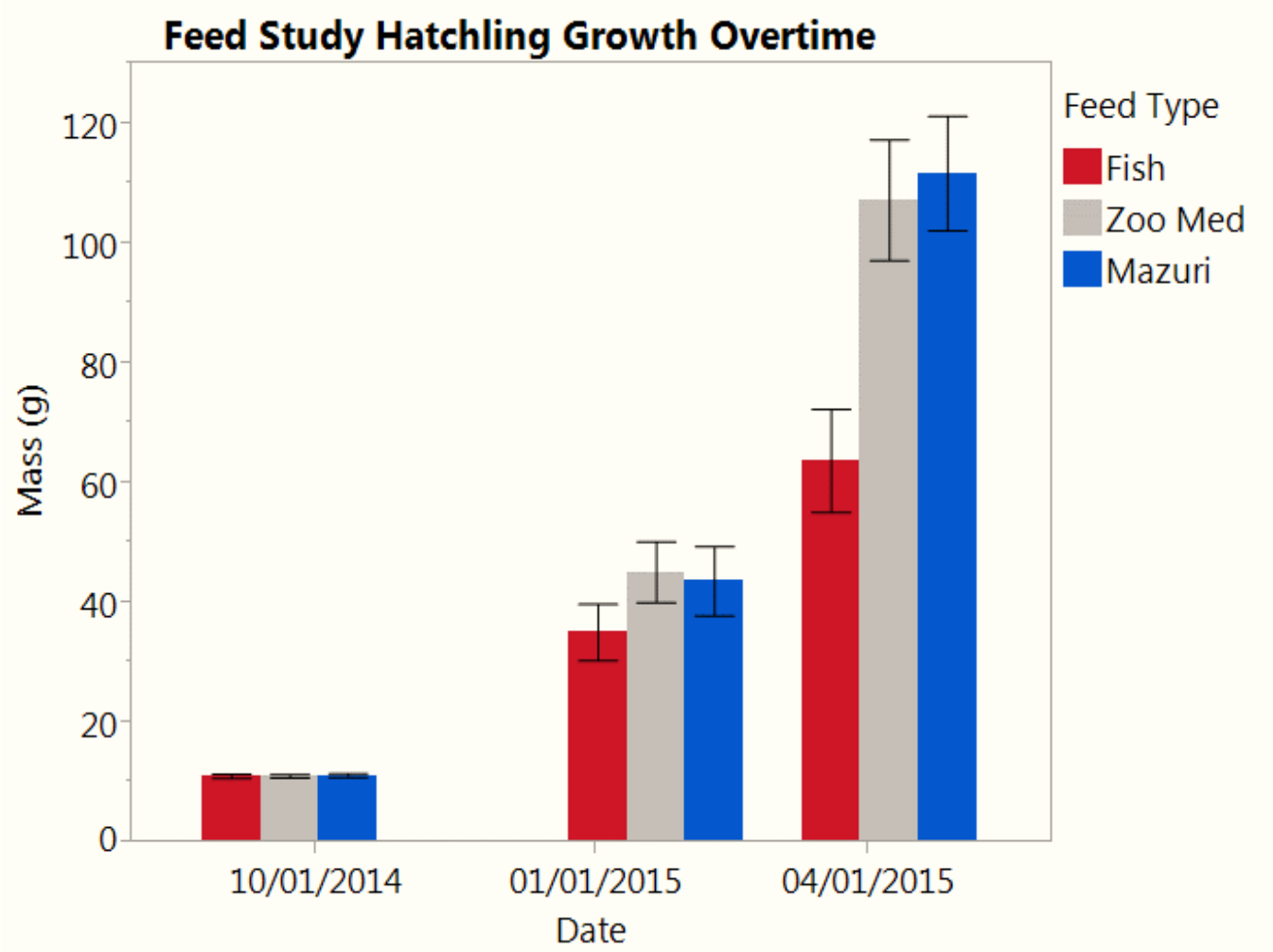

Figure 2. Diamondback terrapin hatchling growth overtime (every 3 months) when offered an exclusive diet of one of three diets (Zoo med commercial pellet, Mazuri commercial pellet or fish filets).

Table 1. Comparison of Gravid Females Captured, Number of Clutches and hatching Success of Females Captured In-Water and on Land.

Female Collection	Total Females Captured	Total Gravid Females	Percent Gravid	Total Clutches	Hatching Success
In-Water	69	60	0.87	60	91%
Land	20	11	0.55	11	85%