Optimal Nest Site Selection for Clutch Relocation at Sandy Point National Wildlife Refuge, St. Croix

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Introduction

During the 2006 leatherback nesting season a preliminary study was performed to observe the effects of respiratory gas composition, temperature, and moisture on leatherback nests. Leatherback nests have significantly lower hatching success than other species of sea turtle at 50-55% (Whitmore and Dutton, 1985) and relocated nests have a significantly reduced hatching success when compared to *in situ* nests (Eckert and Eckert, 1990 Garner et al, 2005). Additionally, a disturbing trend of decreased hatching success has been observed over the history of the project, including a record low of 40.28% in 2005 (Garner et al., 2005). It is believed that factors such as gas, temperature, and moisture have a significant impact on development, and may provide insight into the poor hatch success observed in leatherback sea turtles at Sandy Point,

It has been shown that the rate of oxygen uptake increases rapidly during the second half of incubation in sea turtles (Ackerman, 1981). Given that Sandy Point nests exhibit an increase in late stage embryonic mortality (Eckert and Eckert, 1990) it is possible that oxygen could be a significant factor affecting relocated nests. This is contrary to a similar experiment in Costa Rica in which it was found that nests exhibit early stage mortality and oxygen does not influence embryonic mortality (Wallace et. al. 2004). Temperature is the deciding factor for sex determination in all sea turtle species (Broderick et. al. 2000) and may also function as an influencing factor for hatching success. Research conducted on chelonian eggs has shown that moisture affects the mass of hatchlings, with an increase in moisture attributing to an increase in mass (Tracy et. al.1978). This may produce hatchlings that are more "fit" for survival. This theory is supported by Packard (1999) in which hatchlings with increased moisture exchange grew to a larger size before hatching. These larger well-hydrated hatchlings may survive better than smaller less-hydrated hatchlings. According to Packard and colleagues (1985) experiments with moisture on the terrestrial turtle *Terrapene ornata* determined that moisture does influence egg mass, but does not directly influence hatching success.

In addition to the previously mentioned nest parameters, sand grain size was also investigated. Mortimer (1990) determined that mortality in green turtle (*Chelonia mydas*) nests positively correlates with sand particle size. Sand particle size may be an important factor for embryonic mortality at Sandy Point. It is clear that environmental factors within the nest may significantly impact egg development and mortality. This study served to determine if different sections of the beach exhibit unique characteristics and therefore produce varying hatch success. Additionally, it examined which regions of the beach may provide the optimal nest environment and maximize hatching success in relocated nests. Understanding the factors impacting hatching success will provide vital information necessary to develop effective management strategies and optimize hatchling production at Sandy Point

Methods

During the 2006 nesting season, as in previous years, nests that were subject to erosion were collected for relocation. A sub-sample of 6 nests were chosen at random from within this group and reburied at two-nest sites with a high percentage of nest retention. Each relocation site contained three relocated nests and one *in situ* nest for monitoring. Section 1 was located between stakes 180 and 185, while section 2 was located between stakes 160 and 165. Nests relocated within these zones were spaced at least one meter from surrounding nests to ensure no intra-nest influence. Eggs were collected as the turtle deposited them into the egg chamber, but before the eggs reached sand. All nests were relocated according to protocol derived from Dutton and colleagues (1992) within two hours of deposition and contained any shelled albumin globs (SAG's) (Sotherland et. al., 2003) deposited by the turtle. All yolked eggs and SAG's were counted and the number was recorded during the relocation process. In order to determine any change in egg mass during the incubation period, 20 yolked eggs from each relocated nest were weighed using an Ohaus Scout Pro portable electronic balance prior to reburial.

Sand measurements were obtained by collecting a sand sample in a Ziploc (Gladware) bag prior to the reburial of eggs. Sand was then measured, at a later time, using a 1984 W.F. McCullough sand-gage to determine the particle size of sand at each site. For moisture, sand samples were collected once every 10 days from opposite ends of each site. A hole was dug to nest depth and sand was collected in a Ziploc (Gladware) bag and kept air tight. These samples were then weighed using an Ohaus Scout Pro portable electronic balance before and after drying to determine percent moisture. To dry samples, sand was placed under a standard UL portable reptile heat lamp (Sylvania 75 Watt bulb) for 24 hours.

Sampling ports, consisting of hollow plastic film containers (with Tygon tubing and a thermocouple wire) were placed in the center of each nest before the nest was filled with sand. The film containers served to protect and stabilize the tubing and thermocouple. The thermocouples consisted of a 24-gauge Cu/Cn wire with attachments designed to be read by a BAT 12 thermocouple meter (Physiotemp). A 1 meter length of Tygon tubing extended from the sampling port at the center of the clutch to the sand surface. Shutoff valves at the surface ensured that no additional water or sand entered the nest. An oxygen sensor (Qubit Systems) and a carbon dioxide analyzer (Qubit systems) allowed air to be drawn from the sampling port through the tubing, using a DC pump. This air was sent through a Drierite column to remove water vapor. Samples were then sent past the sensor to record carbon dioxide and oxygen measurements. Samples were taken for approximately 5 minutes during daylight hours (Wallace et. al. 2004) and were taken at the same time, every other day, before nightly patrols. This is the total minimum amount of time that is required to obtain an accurate sample of respiratory gases. This also allowed consistent measurements without any bulk flow effects (Wallace et. al. 2004).

Nests were excavated two days after emergence of the first hatchling and any whole eggs found within the nest were weighed using an Ohaus Scout Pro portable electronic balance, to determine any changes in mass during incubation. Nest contents (including whole eggs, number of live and dead hatchlings and hatched shells) were counted and recorded. After weighing, all whole eggs were also opened to determine the stage of embryonic mortality according to protocol (Eckert and Eckert's 1990).

Hatch and emergence success were determined based on current mathematical formulas (Eckert and Eckert, 1990, Garner et. al., 2005).

Data analysis

Gas and moisture data was analyzed using Logger Pro Software (Qubit systems). Basic statistical analyses were performed using Microsoft Excel data analysis function.

Results

Egg weights for each nest varied considerably (Table 1.). The mean egg weight for all nests pre-burial was 76.24 grams, while mean weight for all nests post-emergence 83.06 grams. The mean difference in weight from pre-burial to post-burial (excluding nest 5) was +7.19 grams. No data was recovered post emergence for nest 5 due to a lack of sample size of whole eggs to weigh. No eggs were weighed for *in situ* nests.

Hatch success for relocated nests ranged from 68.89% to 15.84% (Table 1.) with a mean of 39.0%. Relocated nests at site 1 exhibited a mean hatching success of 39.92%. Relocated nests at site 2 exhibited a similar mean hatching success of 38.19%. The average overall hatch success for all relocated nests was 39.06%. Data was only recorded for one *in situ* nest and the resulting hatch success was 85.96%. The second *in situ* nest was predated and therefore eliminated from the study. Given the small sample size this cannot be considered statistically significant. An ANOVA test was used to compare hatch success among the two nest locations (a $p<0.01$ was considered significant). The resulting p-value of 0.66 indicated no significant influence of nest site on hatching success. The mean overall moisture decrease for both sites was 5.29%. For site 1, the mean percent change in moisture was -4.3% while the mean percent change for site 2 was greater at -10.4%. An ANOVA test was used to determine significance ($p < 0.01$). The resulting p-value of 0.087 indicates no significant difference in moisture change among nest sites. .

Sand particle measurements were 0.5 mm for both sample sites

Table 1. Summary statistics for optimal relocation sites, 2006
Average egg weights, change in egg mass, and hatch success for relocated nests are represented

Turtle ID	Average egg weight pre-burial (g)	Average egg weight post-emergence (g)	Overall change in egg weight (g)	Hatch success (%)
XXZ 110*	59.3	81.97	21.97	68.89
AAR 914*	52.64	90.65	37.41	25.58
AAR 254*	91.05	95.3	4.25	25.3
MK 31	82.92	64.45	-17.47	15.84
MJ 190	78.45	N/A	N/A	65.00
KL 28	93.11	82.93	-10.19	33.72
Mean	**76.25**	**83.06**	**-7.194**	**39.06**

(*Represents nests from site 1)

Discussion

Egg weights varied considerably from the time of preburial to the time of weighing at post emergence. Previous work has shown that there is a positive correlation between relative absorption of moisture and hatchling mass (Tracey, 1978 Packard, 1999). Change in mass does not directly increase or decrease hatch success but may cause the larger, well-hydrated hatchlings to be more “fit” for survival compared to the smaller, less-hydrated hatchlings (Tracey, 1978 and Packard, 199). This is supported by Packard’s (1985) experiment in which it was determined that in the *terrapene ornate* turtle, change in moisture does not significantly increase or decrease hatch success. More statistical tests will be run to determine if there is any significance between moisture content and change in egg weight to either support or negate these theories for Sandy Point. However, in spite of statistical evaluation of this data, no direct conclusions regarding moisture, change in egg weight, and hatch success at the sites can be made due to small sample size.

No significant difference in hatch success between relocation area and hatching success was found between experimental relocation areas. This is supported by the similar means that were observed. However, consideration must again be given to the small sample size from each site. Lack of an effect due to relocation site could also be due to the fact that sand particle size was consistent throughout both sites as well as the fact that both sites were located in the accretion zone. In the future, nest sites that exhibit significantly different characteristics (i.e. one in the accretion zone and one in the erosion zone) will be utilized. Additionally, hatch success may be influenced by the individual turtle. The hatch success of individual turtles used in this experiment will be investigated over the entire nesting season to determine if this could be the case.

Finally, due to equipment failure respiratory gas composition was not successfully measured throughout the incubation period. Next season this factor will be examined again and will help in determining the influence of these gasses on hatch success and embryonic development. Additionally, temperature was not measured due to equipment failure of thermocouples due to nest predation and wire breakage.

Although technical difficulties were encountered in the 2006 season, these experiences served to refine the technology and methods utilized for 2007. With a larger sample size (12 relocated and up to 12 *in situ* per site) and new equipment for the upcoming season, I hope to answer the original questions proposed for this experiment as well as any questions that were raised from the 2006 nesting season. Future research will commence earlier and will utilize different nest sites. Nest site 1 will include stakes 156 through 162 while nest site 2 will include stakes 185 through 191. These areas have significantly different characteristics when compared to the areas used in 2006. In 2006, sites 1 and 2 were very similar in sand grain size and general characteristics. By selecting new plots for 2007, I will be better able to determine if different areas of the beach have a significant impact on the nest environment and ultimately hatch success.

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