# An investigation into maternal impact on hatch success: A nest box experiment

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## Introduction

The Sandy Point National Wildlife Refuge (SPNWR), Sandy Point, St Croix, boasts the largest, most comprehensively studied population of endangered leatherback sea turtles (*Dermochelys coriacea*) in the United States and the Caribbean. The Leatherback Sea Turtle Research and Conservation Project at Sandy Point (managed by WIMARCS), serves to protect and conserve this critical population of turtles. Through innovative research, saturation tagging and nest management techniques, WIMARCS aims to meet the U.S. Fish and Wildlife Service's goal of species recovery by maximizing productivity (as measured by hatching and emergence success rates).

Leatherback sea turtles have the lowest hatch success of any species of sea turtle at around 50-55% (Whitmore and Dutton 1985). Mean overall hatch success at SPNWR has averaged between 40.28% and 67.8% over the past 25 years, with an overall trend of decreasing hatch success (Figure 1.)(Garner et al. 2005). The lowest hatch success in the history of the project was recorded in 2005, with a mean overall hatch success of 40.28% (combining 42.66% *in situ*, and 37.88% relocated) (Garner et al. 2005). As the population of nesting turtles and sample size of nests excavated has increased, a disturbing trend of poor success has been uncovered. In spite of management efforts, this is in direct opposition to the goals of the USFWS recovery plan, as well as WIMARCS recovery goals. The factors affecting the decrease in hatchling productivity and increased mortality at Sandy Point have yet to be determined. Low hatch success in leatherback nests has been determined to be due to high embryonic mortality, rather than infertility (Bell et al., 2003). The embryonic mortality at SPNWR,is most likely due to environmental factors (such as humidity, temperature, water table, salinity, gas exchange, bacteria and sand properties) or maternal factors (such as nutritional status, nutrient availability, and hormonal influences) (Ackerman, 1997). Maternal nutrient availability and subsequent nutrient deposition in the egg yolk have been shown to impact egg/hatchling development, hatch success, immunity, and survivability in many species of birds and reptiles (Thompson and Speake, 2003; Surai and Sparks, 2001; Surai, 2000; Surai et al., 1999; Royle et al., 1999; Cartland-Shaw et al., 1998). The distinct factors involved at SPNWR require further investigation. An investigation into the factors affecting the population's productivity will aid in the modification of management techniques to maximize hatchling production and aid in species recovery.

The impact of maternal investment on hatch (and emergence success) at Sandy Point has yet to be investigated. Hormonal production and nutritional status of the mother will impact the nutrients deposited in the egg for development of the embryo (Thompson and Speake, 2003; Surai and Sparks, 2001 Royle et al., 1999). Changes in maternal status, as evidenced by changing blood values (i.e. lipids. protein, calcium, testosterone, thyroid hormone, etc.) may affect the ability of the embryo to develop or successfully hatch (McGraw et al, 2005; Royle et al., 1999; Cartland-Shaw et al., 1998;). This project was designed to determine the "baseline" success of a clutch (where "baseline success" is defined as the hatch success of a clutch incubated in an optimal environment), and correlate any trends or changes in success with maternal blood values. This will provide valuable information regarding the impact of maternal status on hatch success and population productivity, while increasing our basic understanding of leatherback physiology and nesting biology.

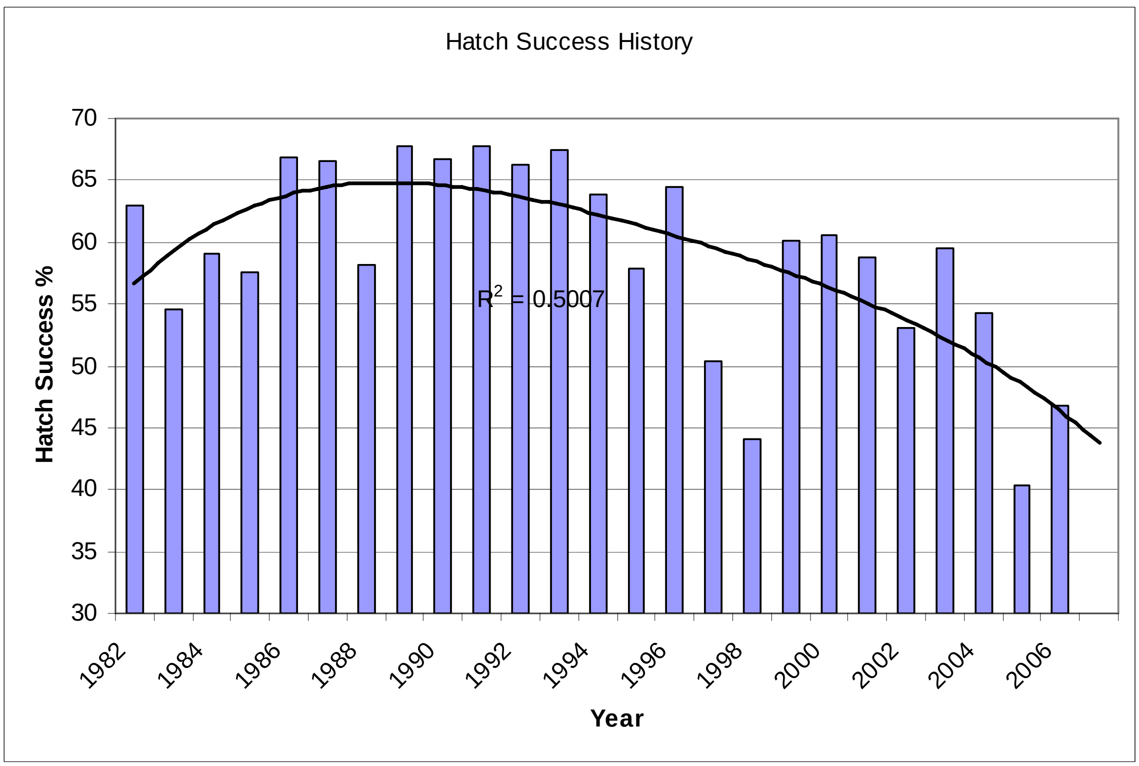


**Figure 1. Comparison of mean overall hatch success for leatherbacks at Sandy Point National Wildlife Refuge (1982 to 2006 nesting seasons).**

## Methods

Nine turtles were selected for inclusion in the maternal impact study. These turtles included 4 individuals identified as neophytes for the 2006 season, as well as 5 individuals identified as new remigrants. Turtles were deemed remigrants by the presence of a previously applied PIT or flipper tag. Turtles were deemed neophytes if no current tags or tag scars were observed. Turtles with no previous record of nesting activity in 2006 were chosen to ensure that all nests could be obtained for artificial incubation.

**Eggs/Hatchlings**

Each clutch from the selected nesters was collected during deposition utilizing standard relocation procedures (Garner, et al. 2005) and artificially incubated as described by Schulz (1975). Upon collection, the yolked eggs were counted and immediately transferred to a styrofoam box lined with a few inches of moist, fresh sand from below the berm at stake 157. Sand from this area was used for all artificially incubated nest boxes in this experiment. Holes were punched in the bottom of each box to ensure proper drainage. A maximum of 60 eggs were placed in a single nest box. If a turtle laid more than 60 eggs, then the remainder were either relocated to a separate nest box, or reburied on the beach. A thermocouple was placed in the center of each nest to monitor temperatures. After all eggs were placed in the box, a layer of moist paper towels was placed on top of the eggs, followed by a few inches of moist sand. Initial nest temperatures were recorded, and each box was labeled with individual turtle I.D., number of eggs, and date, then artificially incubated.

Yolkless eggs (also known as shelled albumin globules, or SAG's), were not incubated with their respective clutches. Yolkless eggs from each clutch were counted and weighed using a standard 500g kitchen scale, then reburied on the beach.

All styrofoam boxes were housed in a ventilated, secure container protected from extreme temperatures (<25°C or >32°C) and predation. Temperatures were monitored using thermocouples inserted into the center of the nest upon relocation to nest boxes. Readings were taken two times daily (using a BAT12, Physiotemp reader), every other day, between approximately 11a.m-1p.m and 6-8p.m. Temperatures were also taken occasionally at mid-afternoon (around 3p.m.). Sand moisture was maintained by spraying nests daily with fresh water. Boxes were elevated at a 10° angle, and holes punched in the bottom of each nest box allowed excess moisture released from the hatching process to drain into pans. Pans were emptied and rinsed a minimum of two times per day.

Upon hatching, the hatchlings were monitored on a daily basis. Once the yolk sac was consumed, the hatchlings were counted, collected and transported to SPNWR for release during evening hours. After all live hatchlings were removed, the nest contents were extracted, counted, and staged per Whitmore and Dutton (1985). All leftover egg (and nest) contents were returned to SPNWR and buried.

**Adult Nesters**

A blood sample was also obtained from the selected individual adult turtles. Samples were collected during egg deposition and utilized standard aseptic phlebotomy techniques (Dutton, 1996). Two blood samples were collected in a single draw from the selected turtles, every time the individuals successfully nested. One sample was collected in either a 5 or 10ml BD Serum vacutainer containing no solution. The second sample was collected in a 5 or 10ml BD vacutainer containing lithium heparin. A sample was obtained for each clutch successfully deposited throughout the nesting season and corresponded to clutches collected for artificial incubation. Samples were refrigerated until centrifugation. All samples were centrifuged (for 10minutes at 3,000 RPM's utilizing a Unico Powerspin LX Centrifuge), and the serum/plasma was pipetted into labeled 2ml cryovials then subsequently frozen for future analysis of blood parameters at Texas A&M University, College Station, Texas.

**Statistical Analysis**

Hatch and emergence statistics for artificially incubated nests were calculated according to standard formulas (See Garner et al., 2006). Significance of results for the 2006 season was determined using the student's t-test, at a level of significance of 0.05.

**Results**

A total of 24 nests were incubated from 9 turtles, including 5 neophytes and 4 remigrants. Additionally, two reclaimed clutches were artificially incubated[1]. One clutch was exposed at the edge of the berm in the 180's, and one clutch was excavated by a nesting leatherback. Both were collected and incubated in conjunction with the maternal impact nests.

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[1] The two reclaimed nests hatched with high success.

Average temperature fluctuation in one day was around 1°C. This is more typical of *in situ* nest conditions, and is much better than the 3-4°C fluctuation observed in past experiments (Dutton, *pers.com.*). Temperatures remained within the "safe" zone of >25 and <35°C, with an average temperature of 28.99°C. The temperatures obtained in this study produced predominantly male hatchlings (Utilizing an estimated pivotal temperature of 29.4 °C (Wibbels, 2003)).

The first nest box (AAV695) emerged on 7/11/2006, with an incubation of 69 days. Average incubation length for nest boxes ranged from 63-69 days, with a mean of 65.62 ± 1.99 days. This is approximately 4 days longer than the average incubation rate of 61.13 ± 2.32 days observed on SPNWR in 2006, and is better than previous experiments which reported a 10-14 day increase in the incubation time of artificially incubated eggs (Whitmore and Dutton, 1985). Hatch success varied greatly among individual turtles, with a minimum of 0% and a maximum of 96.61%. Of the 24 nests collected, 11 nests hatched with greater than 50 % success rate and two nests exhibited a 0% hatch success. Seven nests exhibited very high hatch success (68.33%-96.61%), with 4 of these nests > 85%. Overall hatch success for all clutches was 50.34% ± 30.45%. This increases to 55.37% ± 27.03% with the exclusion of the two 0% boxes. This is significantly higher than the hatch success of relocated clutches incubated at Sandy Point (41.39% ± 24.59%; P = 0.015) but is not significantly different than *in situ* nests (52.73 ± 23.25% ; P = 0.35). The number of experimental nests with low versus high hatch success is comparable to that of both *in situ* and relocated nests incubated on the beach. Approximately 50% of the nests incubated at Sandy Point had a hatch success greater than 46%, while half were less than 46%. The average "high" success of 71.33% ± 19.91% for artificially incubated nests, however, is greater than the 66.1% ± 14.20% (*in situ*) and 64.7% ± 14.29 (relocated) for "high" success nests incubated naturally at Sandy Point although not significant (P = 0.20, and P = 0.10 respectively). The mortality of eggs in the "poor" success nests was primarily associated with undeveloped eggs (approximatelv 16 per nest). This is similar to the average for all naturally incubated nests at SPNWR, which had 15.18 ± 17.27 undeveloped eggs per clutch. Nest box clutches did *not* exhibit the high late term mortality of natural nests which exhibited 11.57 ± 13.2 and 6.4 ± 11.11 (or approximately 18) total late term mortalities per nest. For artificially incubated clutches, an average of 0.61 ± 0.98 full term pipped and 1.5 ± 1.86 full term un-pipped occurred per nest. This equates to approximately two late term fatalities per nest box, and is significantly lower (P<0.01) than naturally incubated nests. Additionally, the artificially incubated nests did not exhibit the difficulty with emergence that is seen on Sandy Point. Emergence success varied by less than 3% from hatch success, and was high at 52.57% ± 25.36%. This is significantly higher than the emergence success for relocated nests (P < 0.01) as well as the overall emergence success at Sandy Point (P = 0.04), which was only 42.42 ± 24.62%. Overall statistics for artificially incubated eggs were very similar to those produced by *in situ* nests, but were significantly better than those produced by relocated nests, as well as the overall average for all nests incubated at Sandy point.

Very few developing embryos (i.e. early and late pigmented eye, early and late pigmented body) were found. Eggs in the nest boxes appeared to develop to full term and hatch successfully, or not develop at all.

There was a high variance among individual hatch success for turtles included in this experiment, and there did not appear to be any significant trend in hatch success over the course of the nesting season ($r^2 = 0.19$). Two turtles, however, did exhibit a consistency in hatch success. Turtle XXZ 160 exhibited low hatch success for all 4 nests (33.33%, 38.98%, 23.53%, and 36.73%), while AAV 695 exhibited consistently higher hatch success for 3 nests (76.67%, 58.62%, and 49.18%), although success declined with consecutive clutches.

The hatchlings produced in this experiment appeared to be very healthy, strong, and active. When compared to hatchlings from many *in situ* and relocated nests incubated on the beach, artificially incubated hatchlings appeared more healthy and hydrated. There were also no instances of kinked, or crumpled hatchlings. Additionally, live eggs and full term pipped eggs had a higher survival rate when compared to live eggs and full term pipped eggs retained from Sandy Point nests (personal observation). There were only 2 fatalities among live eggs, full term pipped eggs, and hatchlings with yolk sacs retained in the lab from nest boxes.

Blood samples are currently being analyzed by the Texas Veterinary Diagnostic Laboratories (TVMDL), College Station, Texas. Results and statistics relating blood values to hatch success will be reported when available.

**Discussion**

This experiment attempted to establish a "baseline hatch success" for turtles nesting at Sandy Point in 2006. "Baseline success" is defined as the hatch success of a clutch incubated in an optimal environment. Incubating nests artificially from day one using optimal environmental conditions served to provide this baseline information. Without major environmental influences, the success of the clutches was deemed to be solely related to maternal investment and impact of maternal factors, such as hormones, on the developing eggs. The high average hatch success of experimental clutches , in addition to the high hatch success of multiple nests ensured that the nest box conditions, including temperature, moisture, and ventilation, were indeed sufficient to promote the successful development and hatching of leatherback eggs. Per this experiment, therefore, the variation in hatch success observed among the turtles was attributed to individual turtle variation, such as nutritional/hormonal status or lack of fertilization, and *not* environmental conditions.

In spite of the optimal conditions, baseline hatch success was very similar to *in situ* nest success for the 2006 season, suggesting that low hatch success in leatherbacks may be maternally derived and innate to the reproductive physiology of this particular species.

There was, however, a discrepancy among all naturally incubated nests (*in situ* and relocated) and the experimental nests, with regards to embryonic mortality. All nests incubated at Sandy Point exhibited significantly higher embryonic mortality. This is due mainly to the high percentage of late term deaths (full term unpipped, full term pipped) observed at SPNWR. It is believed that if the experimental nests were allowed to incubate naturally, hatch success would have significantly decreased due to this phenomenon.

SPNWR has historically exhibited extremely high embryonic mortality, however, this was not observed in the artificially incubated nest boxes. Therefore, this mortality is attributable to environmental factors (such as humidity, temperature, water table, salinity, gas exchange, bacteria and sand properties) during incubation and is *not* due to maternal factors (such as nutritional status, nutrient availability, and hormonal influences). Eggs in the artificial nests either successfully developed and hatched, or were undeveloped. This fact, along with the fact that artificial nests had very few early or late stage embryonic mortalities also supports this hypothesis. The high emergence success observed in nest boxes also suggests that decreased emergence success is a factor of nest construction and environmental factors related to the beach.

One possible factor that may work independently, or in conjunction with those previously mentioned, such as temperature, gas exchange, and nest construction, is clutch size. In order to limit the extrinsic factors affecting egg development., the experimental clutches were limited to a maximum size of 60 eggs per box. Clutch size for nests incubating on the beach, however, varied significantly (from 7-128 yolked eggs), with a mean of 75.74 ± 18.93 eggs (Garner et al., 2006). Evaluation of hatching statistics, in combination with clutch size, shows that clutches containing 60 eggs or less have a significantly higher hatch success (55.05% ± 28.38%; $P = 0.01$) than those with greater than 60 eggs (44.38% ± 23.45). Emergence success is also significantly higher ($P < 0.01$) at 53.23% ± 28.27% versus 39.46% ± 23.19 respectively. This concurs with findings from Culebra, which determined that clutches between 52 and 56 eggs exhibited the greatest hatch success (Hall, 1989). Clutch size may also significantly affect embryonic mortality. Clutches containing up to 60 eggs contained an average of 5.14 full term mortalities (pipped + unpipped) per nest, while those containing 61 eggs, or more, had an average of 21.10 full term mortalities per clutch. Further investigation into the factors affecting nest environment and hatchling emergence during natural incubation is required to understand the phenomenon of embryonic/late term mortality on the beach. This is vital to implementing effective management strategies and maximizing hatchling production.

Additionally, the high average number of undeveloped eggs seen in both naturally incubated nests (12 eggs per *in situ*-17 eggs per relocated clutch) and artificially incubated nests (16 eggs per clutch) suggests that this trait may be innate to leatherback turtles and is exacerbated by the environment. Egg handling and transport on the beach, as well as to the lab most likey impacted the higher number of undeveloped eggs observed in relocated nests, and the proportionally higher number observed in experimental nests. However, it is clear that 12 eggs per *in situ* nest is still very high when compared to other sea turtle species, and appears to be maternally derived. The initial step in future studies is to differentiate between undeveloped and unfertilized eggs, while minimizing the influence of transport. This will be incorporated into studies during the 2007 nesting season.

Overall, this study confirmed that there is a significant impact of the nest environment on hatchling development and emergence success, as well as hatchling viability. The artificially incubated hatchlings were less likely to die prior to, during, or after pipping. They appeared thicker, rounder, and were more hydrated and active when compared to hatchlings produced on the beach. The hatchlings produced artificially appeared healthier and were therefore potentially more "fit" than those produced naturally on the beach. This may be related to increased moisture content in experimental nests, and a resulting increase in hatchling mass (Tracy et. al.1978). A significantly higher hatch success, similar to that of *in situ* nests was observed, along with significantly higher emergence success and significantly decreased embryonic and late term mortality when compared to all Sandy Point nests. Artificial incubation and optimal environmental conditions do not appear to change the overall mean success rate for hatching leatherback turtles, suggesting this, along with the presence of a high number of undeveloped eggs is maternally derived. It does, however, change the pattern of mortality, suggesting that high mortality in nests is related to environmental circumstances, nest construction, and/or clutch size and *not* to maternal fitness. This is an important discovery, since it implies that manipulation of nest environment or construction may serve to eliminate this problem, thus saving an average of 17 turtles per nest. This is a significant number when multiplied by the average number of nests laid per season. In 2006, this would have equated to an additional 6,341 hatchlings. Further research will provide insight into this phenomenon and hopefully provide data for developing effective management strategies to maximize hatchling production and minimize full term mortality.

Additionally, despite the success of artificially incubated nests, there was still extreme variation in hatch success among the experimental turtles. This is attributable to maternal impact on egg development. The large variation in hatch success observed within and among turtles, and throughout the season, suggests that the maternal impact on development is significant and most likely very complicated. A number of factors including maternal hormones and maternal nutrient status may affect egg development and viability (McGraw et al., 2005; Thompson and Speake, 2003; Surai and Sparks, 2001; Surai, 2000; Surai et al., 1999; Royle et al., 1999; Cartland-Shaw et al., 1998). Evaluation of chlorophyll levels in the Atlantic may provide insight into the health of leatherback feeding grounds during the remigration years prior to nesting (Wallace et al, 2006). Leatherbacks feed on a nutrient poor food source, and this, along with changes in food availability may affect reproductive output, and ultimately hatch success in a given nesting season (Bjorndal, 1997; Wallace et al, 2006). Evaluation of blood samples will provide further insight into the maternal factors affecting hatch success, and hopefully provide reasons behind the large variation in hatch success within artificially and naturally incubated nests.

**Literature Cited**


Ackerman, R.A. 1997. The nest environment and the embryonic development of sea turtles. In: The Biology of Sea Turtles. Lutz, P.L. and Musick, J.A. (eds.). CRC Press, Boca Raton, FL, pp.83-106.

Bjorndal, K.A. 1997. Foraging Ecology and Nutrition of Sea Turtles. In: The Biology of Sea Turtles. Lutz, P.L. and Musick, J.A. (eds.). CRC Press, Boca Raton, FL, pp.199-231.

Cartland-Shaw, L.K., A.Cree, C.M. Skeaff, and N.M. Grimmond. 1998. Differences in dietary and plasma fatty acids between wild and captive populations of a rare reptile, the tuatara (*Sphenodon punctatus*). *J. of Comp. Physiol.* 168(8):569-580.

Dutton, P.H. 1996. Methods for collection and preservation of samples for sea turtle genetic studies. Pp. 17-24. *In* Bowen, B.W. and W.N. Witzell (eds.) Proceedings of the International Symposium on Sea Turtle Conservation Genetics. *NOAA Tech. Mem*. NMFS-SEFSC-396. 173 pp.

Dutton, P.H., C.P. Whitmore, and N. Mrosovsky. 1984. Masculinisation of leatherback turtle, *Dermochelys coriacea*, hatchlings from eggs incubated in styrofoam boxes. *Biol. Cons*. 31:249-264.

Garner, J.A., S. Garner, and W. Coles. 2005. Tagging and Nesting Research on Leatherback Sea Turtles (*Dermochelys coriacea*) on Sandy Point, St. Croix, U.S. Virgin Islands. Annual Report to the U.S. Fish and Wildlife Service. *U.S.F.W.S. Pub.* 56p.

Garner, J.A., S. Garner, and W. Coles. 2006. Tagging and Nesting Research on Leatherback Sea Turtles (*Dermochelys coriacea*) on Sandy Point, St. Croix, U.S. Virgin Islands. Annual Report to the U.S. Fish and Wildlife Service. In Prep.

Hall, K.V. 1989. Hatching success of leatherback turtle (*Dermochelys coriacea*) clutches in relation to biotic and abiotic factors. *Proceedings of the Tenth Annual Workshop on Sea Turtle Biology and Conservation. NOAA Technical Memorandum NMFS-SEFC-278.*; 1990, p. 197-200

McGraw, K.J., E Adkins-Regan, and R.S Parker. Maternally derived carotenoid pigments affect offspring survival, sex ratio, and sexual attractiveness in a colorful songbird. *Naturwussenschaften*. 92(8):375-380.

Mrosovsky, N. and C.L. Yntema. 1980. Temperature and dependence of sexual differentiation in sea turtles: Implications for conservation practices. *Biol. Cons.* 18:271-280.

Royle, N.J., P.F. Surai, R.J. McCartney, and B.K Speake. 1999. Parental investment and egg yolk lipid composition in gulls. *Functional Ecology*. 13:298-306.

Schulz, J.P. 1975. Sea turtles nesting in Surinam. *Zool. Verh*. 143:1-143.

Surai. P.F. 2000. Effect of selenium and vitamin E content of the maternal diet in the antioxidant system of the yolk and the developing chick. *British Poulty Sci.* 41(2): 235-243

Surai, P.F., and N.H.C. Sparks. 2001. Comparative evaluation of the effect of two maternal diets on fatty acids, vitamin E and carotenoids in the chick embryo. *British Poultry Sci.* 42(2):252-259.

Surai, P.F., I.A. Ionov, E.F. Kuchmistova, R.C Noble, and B.K. Speake. 1999. The relationship between levels of α – tocopherol and carotenoids in the maternal feed, yolk, and neonatal tissues: comparison between chicken, turkey, duck, and goose. *J. of the Sci. of Food and Agr.* 76(4):593-598.

Thompson, M.B. and B.K. Speake. 2003. Energy and nutrient utilization by embryonic reptiles. *Comp. Bioch. And Physiol Part A* . 133:529-538.

Tracy, R.C., G.C. Packard, and M.J. Packard. 1978. *Water Relations of Chelonian eggs.* The University of Chicago. 378-387.

Whitmore, C.P. and P.H. Dutton. 1985. Infertility, embryonic mortality and nest-site selection in leatherback and green sea turtles in Suriname. *Biol. Cons*. 34:251-272.

Wibbels, T. 2003. Critical Approaches to sex determination in sea turtles. In: The Biology of Sea Turtles. Lutz, P.L., Musick, J.A, and J. Wyneken (eds.). CRC Press, Boca Raton, FL, pp.103-134.