Research Report

Relationship between nest position and predation rates in green sea turtles (Chelonia mydas)

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Summary

Playa Norte is located north of Tortuguero within the Barra del Colorado Wildlife Refuge, bordering the Caribbean sea. The research site consists of a 5km-long transect along the beach that extends from the Tortuguero river mouth to the north end of Laguna Cuatro. As Tortuguero hosts the largest green-sea turtle (*Chelonia mydas*) rookery in the Caribbean, it is important to know the behaviour of the predators that threaten turtle's nests in this area.

Over the last 141 years the global population of the green sea turtle has decreased by 37 – 61%, giving them the status of globally endangered (Troëng and Rankin, 2005). Poaching of eggs and killing turtles for their meat are believed to be the main reasons for the species decline (IUCN red list, 2004; Hart et al., 2013). To get a better understanding of these species, which is beneficial to both researchers and locals, organisations like Caño Palma Biological Station patrol the beach and collect data. This includes data collection on nest success and predation. The goal of this research is to determine if green sea turtle nest predation is related to their vertical position on the beach.

The beach is divided in three zones depending on the percentage of shade the part of the beach gets during the day (0-50% - Open (O), 51-99% - Border (B), 100% - Vegetation (V)). The data has been collected during excavations of the nests of the green turtles within the given transect of beach. These nests have previously been recorded and marked the night the eggs were laid as part of the Caño Palma Biological Station Marine Turtle Monitoring & Tagging Program. The following is a list of predation categories which are used in the study: Micro-organisms, Mammals, Crustaceans and Other. The presence of Ants inside a nest has also been recorded.

Data from a total of 101 nests have been used for this study of which 17 were poached and data was lost. A total of 8385 eggs were counted during excavations, of which 88 were predated by Micro-organisms, 12 by Crustaceans and 114 by Other or unknown form of predation. The majority of these other forms consisted of unknown species of worms. The remaining 8171 eggs were successfully hatched. 19 nests were predated by Mammals and Ants were present in 12 nests. Looking at the percentage of predated eggs in each zone, Vegetation scores highest (8.43%) followed by Open (2.83%) and Border (1.26%).

The Vegetation zone has the most predated eggs in total (8.43%), as well as by individual category: Micro (2.81%), Crustaceans (0.22%) and Other (5.39%). The Two way ANOVA statistical test has shown that green sea turtle nest predation is for the most part related to their vertical position on the beach. Mammal nest predation is most common in the Open zone (21.43%) followed by Border zone (20.75%). Pet and stray dogs have been found a major predation factor on the Playa Norte beach. Almost 19% of all nest recorded during this study have been predated by Mammals of which most, if not all, are dogs. Ants have been found mainly in nests in the Border zone (15.09%), followed by the Open zone (14.29%). Most Ants have been found in mammal-predated nests. As Ants on their own are not able to open eggs, they get access to the insides of an eggs once a mammal destroys the shell (Allen et al., 2001).

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1. Introduction

Over the last 141 years the population of the green sea turtle (*Chelonia mydas*) has decreased by 37 – 61%, giving them the state of globally endangered (Troëng and Rankin, 2005). Poaching of eggs and killing turtles for their meat are believed to be the main reasons for the species decline (IUCN red list, 2004; Hart et al., 2013). Beside these, there are several Other factors that cause the decline in the number of sea turtles. The illegal sea turtle shell trade, marine debris (when ingested can cause turtles to suffocate and drown), artificial lighting (disorientation of hatchlings and nesting females), coastal armouring (loss of available nesting grounds), beach activities (disturbing nesting), predation of eggs by invasive species, pollution, oil spills, climate change and beach nourishment (can drastically change the beach sediment), can all threaten the sea turtle throughout its different life stages (Sea Turtle Conservancy, 2011; Ikonomopoulou et al., 2013; Silva et al., 2013).

The green sea turtle is a reptile and the largest species within the family *Cheloniidae*. It inhabits tropical and subtropical coastal waters around the world, can weigh up to 317,5kg, and reach 1,5m in length (National Geographic, 2013). The green sea turtle is cold blooded, meaning that its body temperature is regulated by the environment (Sea Turtle Conservancy, 2011). Green sea turtles can live for over 80 years and reach an age of sexual maturity at 20 to 30 years old (Zug et al., 2002). Though carnivorous when juvenile, adult green turtles are the only truly herbivorous marine turtles, as they feed mainly on sea grasses and algae (Saenz et al., 2007). Green turtles undertake lengthy migrations from feeding to nesting grounds, the latter being located on sandy beaches (Hart et al., 2013). Mating occurs every 2 to 4 years, normally taking place in shallow waters close to the shore. To nest, females leave the sea and choose a nesting area often on the same beach where they were born (National Geographic, 2013; Hart et al., 2013). They dig a body pit and egg chamber in the sand with their flippers, then fill it with a clutch of 80 to 150 eggs before covering the egg chamber, disguising the nest and returning to sea (Arkive, 2013). The nesting season varies with the locality, and is observed between May and October in the study site (Playa Norte, Tortuguero, Costa Rica) (Arce and Jones, 2010). Nesting occurs with 2 to 4-year intervals. A female may lay as many as 9 clutches within a nesting season (overall average is about 3.3 nests per season) at about 13-day intervals. Incubation ranges from about 50 to 55 days, depending on the temperature (IUCN red list, 2004; Ikonomopoulou et al., 2013; Haysa et al., 2002).

Hatchlings generally emerge at night in groups and find their way to the ocean by natural instinct to move towards light (Ehrenfeld and Carr, 1967). This is the most dangerous time of a sea turtles life, as multiple predators prey on the hatchlings during this small trip to the ocean (United States Fish and Wildlife Service, 2005; Arkive, 2013). Laying multiple clutches during a single nesting season helps to increase the chance of egg survival in the face of unpredictable environmental changes (Ekanayake et al., 2010). Though not confirmed in the green turtle, in the common snapping turtle clutches deposited over time decrease the likelihood that the total breed of a single individual would be lost by a nest predator (Obbard and Brooks, 1979). Thus, multiple clutches may be more important for long-term survival of populations than the total amount of eggs laid (Ekanayake et al., 2010).

Four of the world's seven marine turtle species nest in Playa Norte, Costa Rica: leatherback (Dermochelys coriacea), green (*Chelonia mydas*), hawksbill (*Eretmochelys imbricata*) and loggerhead (*Caretta caretta*). In an effort to protect sea turtles from going extinct, national laws have been established that give the sea turtle significant legal protection in Costa Rica. These laws deem it illegal to harm, harass or kill any sea turtle, hatchling or their eggs (Costa Rican Law 8325). By the same legislation, it is also illegal to import, sell, or transport turtles or their products in Costa Rica. To

get a better understanding of these species, which is beneficial to both researchers and locals, organisations like Caño Palma Biological Station, which was established by the Canadian Organisation for Tropical Education and Rainforest Conservation (COTERC), and the Sea Turtle Conservancy (STC) co-operate by patrolling the beach and collecting data on two different nesting sites in the Tortuguero area. By sharing data and educating the local communities about sea turtle ecology and conservation, effort is made to help protecting sea turtle species for the future generation (Sea Turtle Conservancy, 2011). This includes data collection on nest success and predation. As Tortuguero hosts the largest green-sea turtle rookery in the Caribbean (Fowler, 1979), it is important to know the behaviour of the predators that threaten turtle's nests in this area.
Known predators in the area include crabs, Mammals, birds and Micro-organisms. Pet and stray dogs are known to frequently predate nests (Christen, 2013). Knowing where predators are common gives the Caño Palma Biological Station a better understanding about the beach which they are monitoring and protecting. The goal of this research is to determine if green sea turtle nest predation by these predators is related to their vertical position on the beach.

Beach characteristics are also found to have an influence on the survival of a green sea turtle's nest (Mortimer, 1990). These vertical beach zones (Open (O), Border (B) and Vegetation (V)), are categorised by the percentage of shade this part of the beach gets during the day. The Open beach zone (0-50% shade) has a higher temperature and consists of mainly sand. Temperature and humidity levels fluctuate heavily as there is nothing on the beach which blocks the sun or holds the moisture. The Border zone (51-99% shade) is closer to the Vegetation, which gives the possibility of roots being present. Also due to the amount of shade, temperature can be slightly lower in comparison with the Open beach zone. Also the fluctuations in temperature and humidity are less drastic because of the presence of some Vegetation which allow for some shade and moisture retention. The Vegetation beach zone (100% shade) is in the vegetation where the ground is mainly filled with roots. The Vegetation provides a lot of shade which assumes to make the temperature the lowest of the three beach zones. This makes that the humidity in the soil is of a higher level due to the smaller amount of evaporation. Fluctuations in temperature and humidity are the lowest in this zone. Another difference in the Vegetation zone is the masking of the turtle nest by sight or smell to predators (Spotila, 1987; Hendrickson, 1958).

2. Materials & Methods

2.1 Research site

Playa Norte is located north of Tortuguero within the Barra del Colorado Wildlife Refuge, bordering the Caribbean sea. The research site consists of a 5km-long transect along the beach that extends from the Tortuguero river mouth (10º35’34.4”N - 83º31’28.6”W) to the north end of Laguna Cuatro (10º38’06.9”N - 83º32’31.7”W) (Figure 2.1). Caño Palma Biological Station carries out daily patrols within this transect. Data of predated nest sites has been collected for green sea turtles and used to determine the amount of predation in the nests for the 2013 season (Arce and Jones, 2010).

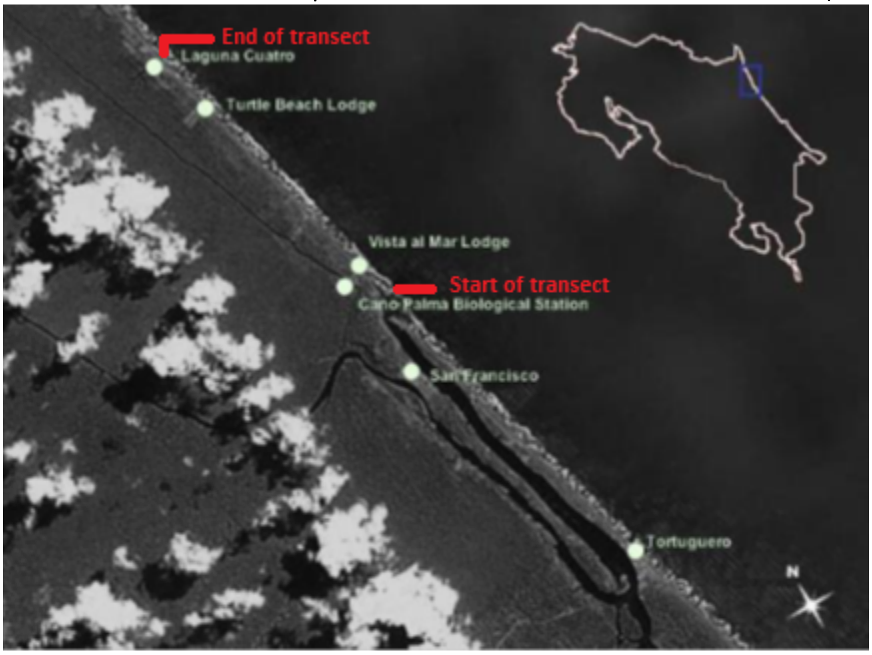

Figure 2.1, Caño Palma Biological Station patrol site (Arce and Jones, 2010)

The beach is divided in three zones depending on the percentage of shade the part of the beach gets during the day (0-50% - Open (O), 51-99% - Border (B), 100% - Vegetation (V)) (Figure 2.2).

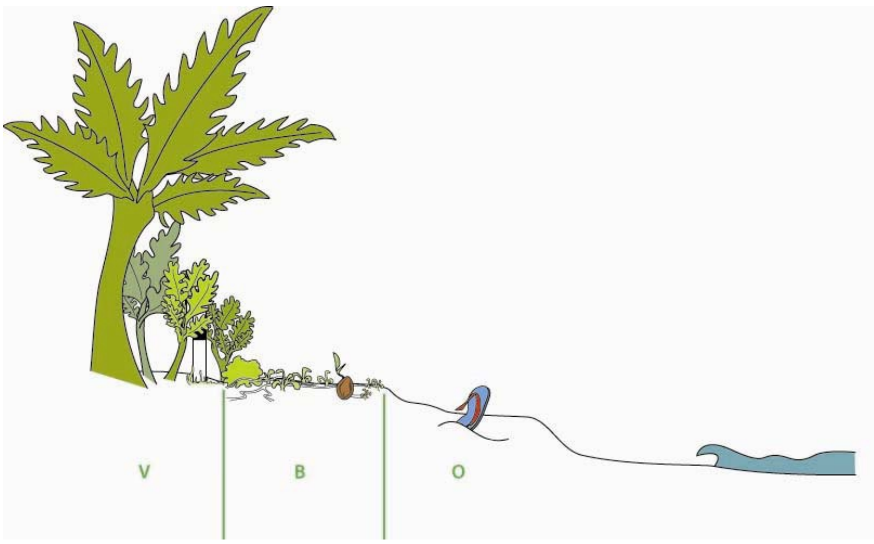

Figure 2.2, Beach zones Open, Border and Vegetation (Arce and Jones, 2010)

2.2 Data Collection

The data has been obtained from green sea turtle nests within the given transect of beach from 5 October 2013 till 31 November 2013. These nests have previously been recorded and marked as part of the Caño Palma Biological Station Marine Turtle Monitoring & Tagging Program the night the eggs were laid. Beach zone (Open, Border or Vegetation) was recorded as part of this data collection. From here on, the nests were monitored for the full incubation period (50-55 days). After day 50, nests were checked for any sign of hatching activity. This was done by checking if there are hatchling tracks or if there is a depression in the sand at the nest location. A depression is a physical sign of hatching activity seen as a slightly lowered layer of sand which is softer then the surrounding sand. A nest was excavated if it has had a depression for five successive days, 2 days after hatchling tracks were spotted or after 65 days without hatching activity. During the excavation, the nest contents were dug up, categorized and recorded in predesigned data collection sheets (Appendix 1). The amount and type of predated eggs were determined based on visual and olfactory cues.

The following is a list of predation categories which are used in the study:

- Micro-organisms

There are numerous causes for an egg to fail to develop, such as flooding (the embryos breathe air through a membrane in the eggs, so they cannot survive if they are continuously covered with water) (Soslau et al., 2011) or temperature fluctuations (Field trip earth, 2012). Without competition, Micro-organisms can develop in the eggs. In this predation category, there will be visual and/or olfactory signs of bacteria or fungi. Although a diagnosis of the exact predator was not possible without a lab, Micro-organisms were recorded as a general category for the purpose of this study. For this reason, these have been grouped together. Bacteria is recognised by signs of bacterial colonies which are spots in either grey, pink or other bright colours inside the dead eggs. Fungi is recognised by a dark green or grey colour and/or spongy formation of spores which have a distinctive mouldy smell (Microbeworld, 2012).

- Mammals

A large amount of locally owned and stray dogs live near Playa Norte. These dogs often dig up nests along the transect, as observed by morning patrols. As the hatchlings emerge from the nest or egg, their smell is picked up by the dogs that can then dig up the nest site, and often destroy the nest and kill remaining hatchlings (Fowler, 1979). Beside dogs there are other mammals that prey on the sea turtles nests. Raccoons are known to cause trouble on sea turtle nesting locations by digging up the nests (Sea turtle conservancy, 2011). Because individual predation is difficult to measure, these are grouped together. A nest which is predated by mammals can be recognised by animal prints, scattered egg shells and a dug up hole at the nest location. Individual egg count is difficult because mammals can eat, destroy or remove the eggs from the nest, so mammal predation is counted by amount of predated nests.

- Crustaceans

Many organisms like flies or ants find it difficult to breach the sea turtle egg shell. Ghost crabs are known to make holes in the eggs to reach the hatchlings inside (Knott, 2009). By these holes other organisms like flies or ants can enter the egg and further predate the eggs (García, 2013). As the crab is the original cause of this type of predation, seen by small holes in the egg, it is the classification used for this predation category.

- Other/Unknown

This study focuses on the predation types mentioned above. All other forms of predation are gathered in one general category called ‘Other’ which also includes unknown predators.

- Presence/Absence of ants

Fire ants may be attracted to disturbance, mucous and moisture associated with turtle nesting , and can establish foraging tunnels into turtle nests shortly after egg-laying (Allen et al, 2001). These fire ants can kill the hatchlings when they start to emerge from the egg (Wetterer, 2007). As it is impossible to diagnose if the ants are the cause of death or attracted by the dead body, only the presence/absence of ants in a nest is recorded in this study. Wetterer (2007) found there are 3 species of ants known for attacking reptiles: red imported fire ant (Solenopsis invicta Buren), tropical fire ant [Solenopsis geminata (Fabr.)], and little fire ant [Wasmannia auropunctata (Roger)] of which the red imported fire ant was by far the most common found in sea turtle nests.

2.3 Processing data

The data of the predated eggs was compared with the vertical beach zone of the nest to find potential connections between the nest site and the predation type. The statistical program SPSS was used to make statistical analyses of the data. The Two way ANOVA test has been used to find potential statistical differences and if found, the Post Hoc test has been used to show between which factors these significant differences are located.

3. Results

3.1 Micro, Crustaceans and Other

A total of 101 nests have been recorded for this study, of which 17 nests were poached. A total of 8385 eggs were counted during excavations of which 88 eggs were predated by Micro-organisms, 12 eggs by Crustaceans and 114 eggs by Other forms. The majority of these other forms consisted of unknown species of worms. The remaining 8171 eggs were successfully hatched. Looking at the percentage of predated eggs in a zone Vegetation scores highest (8.43%) followed by Open (2.83%) and Border (1.26%).

Table 3.1 Total predation of green sea turtle eggs by Micro, Crustaceans and Other

	Total eggs	Micro	Crustaceans	Other
Open	2823	27 (0.95%)	5 (0.18%)	48 (1.70%)
Border	4672	36 (0.77%)	5 (0.11%)	18 (0.39%)
Vegetation	890	25 (2.81%)	2 (0.22%)	48 (5.39%)
Total	8385	88 (1.05%)	12 (0.14%)	114 (1.36%)

By using the Two way ANOVA test a significant difference between predator (Micro, Crustaceans, Other) and zone (Open Border, Vegetation) is found ($F_{4, 213}$=81.699, P=0.000<0.05). A Post Hoc test shows predation between Micro and Crustaceans has a significant difference: Mean Difference = 25.61; Std. Error = 2.549; P= 0.000 < 0.05.
Predation between Micro and Other has a significant difference: Mean difference = -13.15; Std. Error = 1.175; P = 0.000 < 0.05.
Predation between Crustaceans and Other has a significant difference: Mean difference = -38.76; Std. Error = 2.514; P = 0.000 < 0.05.

A Post Hoc test shows zones Open and Border have a significant difference: Mean Difference = 10.34; Std. Error = 1.422; p = 0.000 < 0.05.
Zones Open and Vegetation do not have a significant difference: Mean Difference = -0.88; Std. Error = 1.331; P = 0.509 > 0.05.
Zones Border and Vegetation have a significant difference: Mean Difference = -11.23; Std. Error = 1.442; P = 0.000 < 0.05.

3.2 Mammals and Ants

Of all 101 nests, 19 were predated by Mammals. Table 3.2 shows predation by Mammals is most common in Open zone (21.43%) followed by Border (20.75%) and Vegetation (14.71%). Most, if not all mammal predations have been done by dogs.

Table 3.2 Predation of green sea turtle nests by Mammals

	Amount of nests	Predated nests	Percentage
Open	14	3	21,43%
Border	53	11	20,75%
Vegetation	34	5	14,71%
Total	101	19	18,81%

In 12 nests Ants were present during the excavation. Table 3.3 shows Ants are most commonly found in Border zone (15.09%) followed by Open (14.29%) and Vegetation (5.88%). Ants have been found mainly in mammal predated nests.

Table 3.3, Presence of Ants in green sea turtle nests during excavation

	Amount of nests	Predated nests	Percentage
Open	14	2	14,29%
Border	53	8	15,09%
Vegetation	34	2	5,88%
Total	101	12	11,88%

By using the Two way ANOVA test for Mammals and Ants together also gave a significant difference between predator and zone ($F_{3,5}$=32,750, P=0.030<0.05). A Post Hoc test shows zones Open and Border have a significant difference: Mean Difference = -7.00; Std. Error = 0.816; P = 0.013 < 0.05.
Zones Open and Vegetation do not have a significant difference: Mean Difference = -1.00; Std. Error = 0.816; P = 0.345 > 0.05.
Zones Border and Vegetation have a significant difference: Mean Difference = 6.00; Std. Error = 0.816; P = 0.018 < 0.05.

4. Conclusion & Discussion

Vegetation zone has the most predated eggs in total, as well as in individual categories of predation: Micro, Crustaceans and Other. Mammal nest predation is most common in the Open zone followed by Border zone. Ants have been found mainly in Border zone followed by Open zone. This study shows that green sea turtle nest predation is for the most part related to their vertical position on the beach. The 3 predation types Micro, Crustaceans and Other all show a significant difference from one another, but the zones Open and Vegetation don't. Mammals and Ants together also don't show a significant difference between Open and Vegetation zone.

With these results, Caño Palma Biological Station gets a better understanding about the beach which they are monitoring and protecting. It gives a better idea of where predation is most common. It also shows factors which are important for future turtle seasons. Pet and stray dogs have been found a major predation factor on the Playa Norte beach. Almost 19% of all nest recorded during this study have been predated by mammals of which most, if not all, are dogs.

Nest predation on Playa Norte is for the most part related to their vertical position on the beach which is supported in another study (Fowler, 1979), which found predation to be linked to their vertical position on the beach. Micro has shown to be the highest individual predator inside a nest. This may be caused by the fact that turtle eggs exchange gases and water across their pore containing shells (Ackerman and Prage, 1972) that under some conditions can serve as a portal for the entry of pathogenic bacteria and fungi. Pathogenic bacteria can presumably pass through pores (Al-Bahry et al., 2009; Diaz et al., 2006). Also, fungi of the genus Aspergillus have been identified growing on green turtle eggs and have been postulated to contribute to altered embryonic development and egg mortality (Elshafie et al., 2007). Once one egg is infected the micro infections can be passed from one egg to another (Phillott and Parmenter, 2001). Predation by Crustaceans was found to be a minimal threat to the sea turtle eggs. Ali and Ibrahim (2002) came to the same conclusion with their study of crab predation on sea turtles resulting in 1.3% of eggs predated by crabs.

As pet and stray dogs showed to be a major predation factor during this study, teams have been building protection for the nests made out of logs. Without this, the number of mammal predated nests might have been even higher as this has affected the number found in this study.
Mammal and Ant results have been put together during the analyses. This was decided because of the slim numbers of results found in this study. The similarity between these 2 predation types, as ants mainly have been found in mammal predated nests, might be caused by the disturbance of the nest by mammals which attracts the ants (Allen et al, 2001). Predation type 'Other' was most common in predated eggs. This was influenced by 2 nests with high numbers of unknown predated eggs. This unknown form of predation was mainly caused by worms found inside the eggs. It seems these worms can penetrate the sea turtle egg shell as there were no holes created by crustacean. Further research is needed to confirm this hypothesis.

Dog have been found digging up nests the moment a nest was marked for depression checks, even if the nest turned out to be poached during the excavation. This may indicate that the dogs are learning where the nests are according to the markers that Caño Palma Biological Station uses, though this is not supported in other research (Antworth et al., 2006) which did not found any evidence of predators using markers to find eggs which they have been using for 19 years. For future turtle seasons it is advised to keep building protection against the pet and stray dogs. Although it is not a full protection against the mammal predation, it has shown to be an effective method to keep most of the dogs out. Antworth et al. (2006) found that increased effort to protect nests led to a lower predation rate

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Appendix

Appendix 1: Excavation data sheet

Excavation Date:			MP & Names:	
Nest ID:			Species:	
Laying Date:			Mile:	
Empty Shells (>50%):		Pipped Eggs:		Yolkless:
Unhatched Eggs				
No Embryo:	Stage 1:	Stage 2:	Stage 3:	Stage 4:
Predated Eggs:	Micro:	Crustaceans:	Mammals:	Other:
Deformed Embryos				
Albinos:	Twins:		No Eyes:	Others:
Hatchlings				
Live:			Dead:	
Nest destroyed by another turtle Y/N:				
Egg Depth:			Nest Depth:	
Comments:			Tape found:	

Empty shells (>50%): All eggs pieces larger than 50% of a total egg counts for 1 empty shell.
Pipped Eggs: Hatchling has started to emerge from the egg but died in the process.
Yolkless: Tiny egg without any yolk inside.
No Embryo: Unfertilized egg.
Stage 1: 0-25% of the space inside the egg is taken by the embryo.
Stage 2:25-50% of the space inside the egg is taken by the embryo.
Stage 3: 50-99% of the space inside the egg is taken by the embryo.
Stage 4: 100% of the space inside the egg is taken by the embryo.
Nest destroyed by another turtle Stage Y/N: A second nest laid on top of the original one.
Egg Depth: The depth in cm from the top of the nest to the eggs.
Nest Depth: The depth in cm from the top of the nest to the bottom of the nest.
Tape found: Tape with the nest ID written on it that has been put into the nest during egg count the night the nest was laid.