Effect of temporal flooding on the hatching success of leatherbacks (*Dermochelys coriacea*).

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This report was written under the instructions of the Canadian Organization of Tropical Education and Rainforest Conservation (COTERC) with the purpose of getting a better understanding of the effects that flooding has on the embryonic development of leatherback sea turtles (*Dermochelys coriacea*), which is a phenomenon that has been causing more and more problems for this already vulnerable and declining species. Although gaining knowledge on these kind of problems is vital, having to collect data on such a grim subject under not always favorable conditions can bring anyone down. Which is why without the help of certain people this study could not have been made possible, and I would like to take this moment to thank them. First I would like to thank COTERC staff members Charlotte Foale, Luis Fernández, Helen Pheasey and Emily Khazan not only for their guidance, but also for sharing their expertise on not only sea turtles, but also all the other aspects one has to deal with while doing research. I would also like to thank Sander van Huijzen for both the guidance he gave me during my internship and the help he provided on the statistical analysis of my research. Last but not least I would like to thank all the other volunteers and interns that helped collect data on my research subject, which helped me out immensely.
ABSTRACT

Leatherback sea turtles (*Dermochelys coriacea*) are a vulnerable species that have recently been declining. One of the problems they face is that the increase of the high tide line paired with the leatherbacks preference of nesting on open parts of the beach has caused nests to get flooded. This flooding of nests does however not always mean that eggs do not hatch. In order to get a better understanding of the influence of flooding on hatching success we looked at which stages of the embryonic development eggs are more vulnerable to flooding. To do so leatherback nests were monitored on the Playa Norte beach in Costa Rica from 2013 to 2015. Nests that had been found during this study were monitored daily for signs of flooding and at the end of their incubation period excavated to determine and classify the eggs found with embryos in the different stages of their development. These different stages were then compared against the amount of days a nest was recorded as flooded and when this flooding first started to see if there was any relation between flooding and embryonic death. During the 2015 nesting season nests were flooded so severely on the study site that no data could be collected for this study on any leatherback nests during that season. Results for the data collected in the years 2013 and 2014 show however that embryonic death caused by flooding is more likely to occur in the earlier stages of development. Because of the relatively small sample size and influence of other factors that can cause embryonic death it is however recommended that more research should be done on this subject. It is also recommended that further studies on this subject should focus on bigger study sizes done on beaches with bigger populations of nesting leatherback sea turtles, span over longer periods of time and also closely monitor the changes in the high tide line and other factors that could affect embryonic death over these periods.
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1 INTRODUCTION

Leatherback sea turtles (*Dermochelys coriacea*) are listed as vulnerable (IUCN, 2015) and like most other sea turtle species their populations are declining (Sarti Martínez 2000; Saba et al., 2012; IUCN, 2015). This decrease in the worldwide population of sea turtles can be traced back to human influence in the forms of habitat destruction, poaching, fisheries bycatch and marine pollution (Pritchard, 1982; Spotila et al., 1996; Mrosovsky et al., 2009; IUCN, 2015). In order to halt this decrease more research is necessary to get a better understanding of the more important aspects that can influence sea turtle populations, like size growth (Price et al. 2006, Jones et al. 2011), reproductive output (Price et al. 2006), remigration (Shillinger et al., 2008) and age structure (Broderick et al., 2003). However little is known about these aspects because sea turtles are inaccessible during most of their lifespan (Hirth 1980; Pritchard & Trebbau 1984; Eckert, 2002). When sea turtles nest however they can be found on land where they are more easily approachable. For this reason most of the research done by conservation organizations has been focused on nesting sea turtles. One of these organizations is the Canadian Organization for Tropical Research and Rainforest Conservation (COTERC), which runs the Caño Palma Biological Station. This station has been running a sea turtle monitoring program since 2004 with some initial guidance from the Sea Turtle Conservancy (STC). The station’s research focuses on the sea turtle population of Playa Norte, a beach on the north-east coast of Costa Rica. On this beach four sea turtle species are known to nest, including the leatherback sea turtle.

Leatherbacks were once the most abundant sea turtles in the world (Pritchard, 1982). Their nesting period, for the Northwest Atlantic Ocean subpopulation, starts at March and ends at June and their nests have a mean incubation period of 65 days. With an average carapace length of between 130 and 180 centimeters (cm) and an average weight of between 300 and 500 kilograms (kg) it is also the largest sea turtle (Spotila, 2004). It can easily be distinguished from other sea turtles because it lacks a bony carapace and instead of scutes has a thick, leathery skin and seven distinct ridges that rise from the carapace, crossing from the anterior to the posterior of the turtle’s back. The carapace is colored dark grey to black, with a scattering of white blotches and spots. The turtle’s underside is lightly colored (Sea Turtle Conservancy, 2011). Indications of the presence of leatherback sea turtles on the beach come in the form of tracks, which have an average width of between 150 and 230 cm and are always symmetrical, and in the form of nests, which are large in diameter accompanied by a lot of sand spray thanks to the turtles attempt to cover up the nest (Spotila, 2004).

Apart from the general problems all sea turtles face, leatherbacks in particular face an increased risk due to their preferred nesting location, which is mostly on open parts of beaches just above the high tide line (Mrosovsky, 1983; Whitmore & Dutton, 1985; Kamel & Mrosovsky, 2004). Because of this the nests are more prone to flooding, especially since the recent increase in sea level most likely caused by climate change (Patino-Martínez et al., 2011). The flooding of a nest can cause not only asphyxiation of both eggs and hatchlings through lack of oxygen, but also salinity poisoning, which is negatively affecting the leatherback’s already low hatching success (Bell et al., 2003). However long term monitoring at Playa Norte has shown that flooding doesn’t necessarily mean that nests won’t successfully hatch. Depending on what stage of the incubation the nests are in embryos seem to be more or less susceptible to interference of outside factors that can cause embryonic death (Barbara et al., 2004), including
flooding. There is however little known about at which stage there is a higher risk of embryonic death caused by flooding.

The purpose of this study is to deduce whether leatherback embryos are more susceptible to flooding in different stages of their development. To do so leatherback nests were monitored daily and classified depending on their conditions over their entire incubation period. Through the excavations of the nests at the end of the incubation period the hatching success and the different amount of eggs still present in different embryonic stages were then recorded. This data was then also compared to the amount of days nests have been recorded as flooded and the first day nests have been recorded as flooded to determine whether the severity or the time of the flooding of nests might also play a factor in the nest success. By doing this we hoped to get a better understanding of the effect that temporal flooding can have on the hatching success of leatherback sea turtle nests.
2 MATERIAL AND METHODS

2.1 RESEARCH SITE

This study took place along a 3 1/8 mile transect on Playa Norte (Figure 2.1). The transect stretches from the Laguna Tortuguero river mouth (Datum WGS84 552224.9E 1170322N) to Laguna Cuatro (Datum WGS84 550043.7E 1175989N). The transect, along with the rest of Playa Norte, is part of the “Barra del Colorado” Wildlife Refuge and the southern part borders with Tortuguero National Park. The area is managed by the Tortuguero Conservation Area (ACTo) and is regulated by the Costa Rican Ministry of Environment and Energy (MINAE).

Figure 2.1. Study site with the transect highlighted in red (adapted from Grant & Lewis, 2010).

To define the spatial distribution of nesting activity permanent wooden mile markers at every 1/8 of a mile are used as location markers along the transect. At the same time the beach is divided vertically into three sections, defined by the amount of shade nests will receive. These zones are open with >50% exposure to direct sun light, border with <50% exposure to direct sun light and vegetation with 0% exposure to direct sun light (Figure 2.2).

Figure 2.2. Vertical beach zones where zones with >50% exposure to direct sun light (Open), zones with <50% exposure to direct sun light (Border) and zones with 0% exposure to direct sun light (Vegetation) are pictured (Pheasey & Fernández, 2014).
2.2 Night patrols

In order to collect data on all nesting sea turtles and to find nests to monitor we patrolled the transect in teams each night for the duration of the entire nesting season. For this study, only data related with leatherback nests was taken into account. Patrol teams counted the eggs of any leatherback turtle if at the time of encountering she had not yet started laying her eggs. We counted both fertilized and unfertilized (i.e. yolkless) eggs by placing a hand, while using medical latex gloves, under the cloaca. We used an egg-counter to count fertilized eggs while yolkless eggs were counted by memory. We also measured the exact egg chamber location by triangulation in order to be able to re-locate them for monitoring during their incubation period and for the excavations at the end of the incubation period. We picked three points with at least 45 degrees between them in the vegetation at which we tied flagging tapes with the turtle ID number and the appropriate direction compared to the egg chamber (south, center or north). We then measured the distance between these points and the egg chamber; and between the egg chamber and the high tide line, with a 50m measuring tape (Figure 2.3). Besides these measurements we also recorded the location data (mile marker, vertical zone, GPS coordinates and accuracy of those coordinates) for each triangulated nest.

![Figure 2.3. Schematic overview of a nest triangulation (Pheasey & Fernández, 2014).](image)

2.3 Morning census

To monitor triangulated nests for flooding during their incubation period we also patrolled the transect daily in the morning at first light. During these morning censuses we checked all triangulated nest for any signs of flooding, as well as any other changes from the natural state of nests for the overall data collection. During these morning censuses we also checked the transect for any other signs of sea turtle activity. In case a new nest was successfully triangulated during the night patrols we would also re-triangulate it during the first following morning census to confirm the measurements. If a nest reached its 60th day of incubation we would re-triangulate it again in order to put up depression sticks, three sticks that mark the exact location of the egg chamber, to facilitate the assessment of any signs of hatchling activity. Signs of hatchling activity included a physical depression in the sand around the nest area.
caused by hatchlings digging their way to the surface inside the nest. Hatchling tracks leading away from the nest may have also been present. We monitored all successfully triangulated nests daily until they were ready to be excavated.

2.4 Excavations

To determine whether triangulated nests were successful excavations were performed. The nest’s success only comprised of the hatching success, i.e. the number of eggs that hatched, and not the emerging success, i.e. the number of hatchlings that were able to leave the nest. We only excavated nests under the following circumstances:

1. If hatchling tracks were present, nests were excavated two days later.
2. If there were five consecutive days of depression, nests were excavated on the following sixth day.
3. If no signs of hatching were present after 75 days, nests were excavated on 75th day.

During the first stage of the excavation we needed to locate the egg chamber of the nest by re-triangulating it. We then then carefully removed the sand using a cupped hand until the first signs of the nest appeared (e.g. eggs, empty eggshells or hatchlings). At that moment we took the egg depth from the top of the nest using the bottom flat part of a stick lying over the entrance. We then started to remove and sort the nest contents into five different stages of embryonic development: first there was the no embryo stage for eggs were no embryo is present; stage 1 consisted of eggs where the embryo occupied ≤ 25% of the egg and could be as small as a spot of blood within the yolk; stage 2 consisted of eggs where the embryo occupied 26-50% of the egg; stage 3 consisted of eggs where the embryo occupied 51-75% of the egg, and stage 4 consisted of eggs where the embryo occupied > 75% of the egg (Figure 2.4). When all the contents of the nest had been removed we measured the nest depth from the bottom of the nest to the surface of the beach again using a horizontal stick over the egg chamber for reference.

![Figure 2.4. Different stages with from left to right: stage 1; embryo occupies ≤ 25% of the egg, stage 2; embryo occupies 26-50% of the egg, stage 3; embryo occupies 51-75% of the egg and stage 4; embryo occupies > 75% of the egg (Pheasey & Fernandez, 2014).](image)

From the excavated nests we recorded the amount of eggs found in each different stage. In order to determine if there was a relationship between flooding and the hatching success we then regressed the number of days a nest was recorded as flooded and the first day a nest was recorded as flooded onto the number of eggs found in each stage. To statistically analyze any correlations between nests that were successful/unsucessful and the first day of flooding we also used a general linear model. All analysis were performed by using IBM SPSS statistics (version 20).
3 RESULTS

3.1 OVERVIEW

During the leatherback nesting seasons of 2013, 2014, and 2015 a total of 52 nests were successfully triangulated on the study site. For this study we only looked at nests that had not been recorded as being eroded, nests that where successfully excavated, nest with eggs left and nests that got recorded as being flooded at least once during their incubation period. Hatchlings of these nests that were able to successfully hatch were recorded at 7 nests in 2013, 6 nests in 2014 and 0 nests in 2015 for a total of 13 successful nests (Table 3.1).

Table 3.1. Overview triangulated nests over the duration of this study, were nest that were recorded as eroded have already been excluded from the successful/ unsuccessful nests.

<table>
<thead>
<tr>
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<th>2013</th>
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<td>Leatherback nests</td>
<td>31</td>
<td>14</td>
<td>7</td>
<td>52</td>
</tr>
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<td>Flooded/wet nests</td>
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<td>10</td>
<td>7</td>
<td>42</td>
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<tr>
<td>Eroded nests</td>
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<td>1</td>
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<td>Unsuccessful nests</td>
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<td>4</td>
<td>4</td>
<td>21</td>
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Because of the severity of the flooding seen in the 2015 nesting season no nests were successfully excavated in that year, which is why only data of the 2013 and 2014 nesting seasons could be used to compare differences between embryonic stages in flooded nests. When looking at the average number of days nests had been recorded as flooded between the different years it shows that the year 2015 was a distinctly wetter year. While nests in 2013 only spent an average of 2,86 days and nests in 2014 an average of 5,61 days recorded as flooded nests in 2015 spend an average of 41 days recorded as flooded (figure 3.1).

Figure 3.1. Mean amount of days nests spent recorded as flooded compared between the different nesting seasons.
For the difference between the successful and unsuccessful nests between first days of flooding not much difference can be seen between the different ranges. First days of flooding ranged from day 2 to day 74 for successful nest and day 3 to day 73 for unsuccessful nests, a bigger difference is seen between the average differences between both groups, which were 40.65 (df=13, ±26.78) for successful and 28.58 (df=20, ±22.84) For unsuccessful nests, even though this difference was not significant (p=0.830) (Figure 3.2).

![Figure 3.2](image)

Figure 3.2. Mean first day of being recorded as wet or flooded compared between successful and unsuccessful nests taken from all the seasons.

### 3.2 FLOODING COMPARED BETWEEN EMBRYONIC STAGES

For the unsuccessful leatherback nests of the seasons 2013 and 2014 there where no eggs found in stage 3 or later. For the other stages a range of between 2 and 31 eggs for the no embryo stage, between 1 and 73 eggs for stage 1 and between 1 and 52 eggs for stage 2 where found. In the successful group eggs have been found in all the different stages. With ranges of between 3 and 67 eggs for the no embryo stage, between 1 and 7 eggs for stage 1, between 1 and 3 eggs for stage 2, between 1 and 9 eggs for stage 3 and between 4 and 5 eggs for stage 4 have been found. When looking at the mean of each groups a big difference can be seen between successful and unsuccessful nests at stages 1 and 2 (Figure 3.3). An average of 30,57 eggs with stage 1 embryo’s where found in unsuccessful nests against 1,39 in successful ones. For stage 2 embryo’s an average of 12,00 eggss where found in unsuccessful nests against 0,83 eggs in successful ones.
When regressing the different stages to the first day of flooding it shows however that there is only a significant relation with the no embryo stage ($p=0.038$, $r^2=0.321$). For the amount of flooding it shows there is only a significant relation with the no embryo stage ($p=0.020$, $r^2=0.373$) and stage 2 ($p=0.020$, $r^2=0.373$).
4 DISCUSSION AND CONCLUSION

The year 2015 was not a very productive year for nesting leatherback turtles on Playa Norte. Of all the nests that had been successfully triangulated and monitored during this nesting season not a single nest produced viable hatchlings. This increase in flooding has not only caused more nests to fail but also made it impossible in most cases to determine what stage eggs with embryonic death were in because nests got completely washed away. Most of this increase in flooding can be contributed to the rise of the high tide line over the last few years. Reasons why the high tideline has increased in the last years can be explained by a few things. One of the reasons might be the change in the dynamism of the sea caused by climate change (Patino-Martinez et al., 2011). Other studies have shown that this has also been effecting other nesting leatherback populations (Caut et al., 2010). Another possible factor that could have affected the rise of the high tide line is the increase in storm activity that was seen in study site during the season of 2015. Although this increase storm activity might also be related to climate change storms can also increase the rise of the high tideline by themselves in the form of wind-driven coastal surges and changes in atmospheric pressure (Bunya et al., 2009).

Data from the 2013 and 2014 seasons shows that embryonic death seems to occur more in the earlier stages for both successful and unsuccessful nests. In the unsuccessful nests no embryos were found in stages 3 and 4, and lower amounts of eggs with dead embryos in stages 3 and 4 of successful nests. This suggests that embryonic dead caused by flooding occurs mostly in the earlier stages of development. These results correspond with findings from another study on embryonic death (Barbara et al., 2004). But seeing how that study focuses on embryonic death in general, and the data collected in this study comes from a relatively small study size with few significant findings it can’t be said with certainty that these findings are the result of flooding alone. This could mean that other factors are partly or completely responsible for the embryonic death instead of just flooding. Other possible factors that might be responsible for the embryonic death are predation, temperature changes and bacterial and pest infestations (Wyneken et al., 1988; Barbara et al., 2004). Something else to consider is that this could mean that the effect that flooding has doesn’t immediately occur, and may just slowly progress. Although without further research on this subject this would be impossible to say.

In conclusion it shows that the effect that flooding has on embryonic development of leatherback sea turtles is a complex subject that requires a robust dataset which also includes measurements on other factors (e.g. temperature) in order to fully understand the effect that flooding can have. Seeing as flooding does not only cause embryonic death but also tends to wash away nests completely study size on this subject has to be quite substantial in order to collect enough data to get significant results. With the rise of sea levels and the tendency of nesting close to the high tide line by the already vulnerable and declining leatherback sea turtles research on this subject is however very necessary to get a better understanding, and to find solutions to this problem. This is why we recommend that more studies are done on this subject. These studies should also possibly focus on bigger study sizes done on beaches with bigger populations of nesting leatherback sea turtles. It is also recommended that any further studies on this subject should span over longer periods of time, while also closely monitoring the changes in the high tide line over these periods.
REFERENCES


