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Temporal Trends in the Hatching Activity of Leatherback Sea Turtles on the West Coast of Puerto Rico from 1992-2008

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by

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ABSTRACT OF THE THESIS

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The leatherback sea turtle (*Dermochelys coriacea*), a species considered vulnerable by the International Union for the Conservation of Nature, is found nesting on the island of Puerto Rico. In this study, nesting and hatching activity were analyzed for the first 17 years of collected data, 1992-2008, from El Balneario beach in the town of Añasco on the west coast of Puerto Rico. These data were obtained from Department of Natural and Environmental Resources (DNER), a strand of the government of Puerto Rico. The study data were separated into two bins of the first and last years (bin 1: 1992-1997, excluding 1996, and bin 2: 2004-2008). There were significantly more nesting events (i.e., act of a sea turtle burying its eggs) in bin 2 than in bin 1. However, significantly more eggs hatched and more sea turtles emerged from the nest to the sand in bin 1 than in bin 2. Nest disturbances (poaching, light pollution, and compacted sand) did not significantly affect the percentage of eggs that successfully hatched or emerged in a nest. The likelihood of a nest to have zero hatched eggs was higher in nesting areas disturbed by light pollution. Studies on the results of conservation efforts on sea turtle reproduction have not been conducted at many of the breeding

locations in Puerto Rico. The conservation efforts by the government and community may have increased nesting events. The DNER worked on educating and involving the community in monitoring efforts by encouraging them to volunteer and become stewards of the sea turtles and the nesting beach. For example, at the beginning of the project the community would not report activities that were detrimental to sea turtles or hatching and nesting events. In contrast, during the last years of the study, the community was heavily involved in educating other community members, the monitoring of the beach for sea turtle activities and reporting negative anthropogenic factors that can impact sea turtles. Lower numbers in hatching and emergence success may be attributed to effects on egg development created by a potential increase in the presence of fungi (*Fusarium solani*) and bacteria (*Serratia marcescens*), as well as temperature and humidity in the nest clutch.

The thesis of Fabiola B. Torres-Toledo is approved.

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INTRODUCTION

Several populations of sea turtle species are in decline, with many species being threatened/vulnerable or endangered (Seminoff 2004, Abreu-Grobois and Plotkin 2008, Mortimer and Donnelly 2008, Wallace et al. 2013, Casale and Tucker 2017, Wibbels and Bevan 2019). Anthropogenic activity has heavily contributed to the decline of sea turtle populations (Bjorndal and Jackson 2003). While some populations are beginning to recover, others continue to decline. However, it has been shown that conservation practices have helped to increase sea turtle nesting populations (Dutton et al. 2005). There is hope that with continued conservation efforts, sea turtle populations may recover. To aid in population recovery, it is essential to analyze population trends of sea turtle reproduction (Hamann et al. 2010). Through such analysis, the efficacy of conservation efforts can be assessed in order to develop better management and conservation strategies.

The U.S. Endangered Species Act of 1973 was enacted to control activities that can cause a critical drop in populations of endangered species that contribute to maintaining an ecological balance of our ecosystems such as sea turtles (Wilson et al. 2010). Despite associated protection measures, sea turtles are still considered globally either threatened/vulnerable or endangered. Recovery of sea turtle populations is greatly affected by the ability of sea turtles to access reproductive sites and establish nesting populations. Documenting nesting activity has been a leading method of surveying the health of sea turtles and the assessment of conservation and management practices and is used to determine the status of a species for the International Union for the Conservation of Nature (IUCN) Red List (Wallace et al. 2013). Human activity has been identified as a significant reason for the decline of sea turtle populations with many activities directly impacting sea turtle reproduction (Bjorndal and Jackson 2003). Limiting human impacts on sea turtles could help to recover species abundance (Eckert 2001).

Previous studies have shown that sea turtle conservation efforts, such as nesting beach protection and egg relocation, have helped to increase sea turtle nesting populations which should result in the growth of the population as a whole (Balazs and Chaloupka 2004, Dutton et al. 2005, Antworth et al. 2006). However, these studies have been limited geographically and in the dearth of studies published related to these topics. Studies on the results of conservation efforts on sea turtle reproduction have not been conducted throughout all the breeding locations within North America (Joglar et al. 2007). Therefore, there is a need for knowledge on the status of certain nesting populations to be able to properly focus conservation efforts and management practices on sea turtle nesting beaches. Due to this issue, the focus of this study is based on the reproductive biology of the leatherback sea turtle (*Dermochelys coriacea*) on the island of Puerto Rico.

The leatherback sea turtle is considered by the IUCN to be a vulnerable species worldwide (Wallace et al. 2010). The Northwest Atlantic leatherback sea turtle (*D. coriacea*) visits the island of Puerto Rico to forage and nest (Rivero 1978, Eckert et al. 2012, Eckert and Eckert 2019). The government of Puerto Rico protects sea turtles under US protection laws (Lacey Act of 1900, U.S. Endangered Species Act of 1973). Another way the population of sea turtles is protected and monitored on the island is through rehabilitation, stranding, and educational projects (Moore et al. 2007, Olivares 2016, DNER 2020). Nesting and hatching activity data of the leatherback sea turtle specifically from a beach on the west side of the island of Puerto Rico was used and analyzed as part of this study.

In the town of Añasco, on the west side of mainland Puerto Rico, El Balneario has been the most heavily monitored of all beaches on the west coast (Blas et al. 2014, DNER 1992-2008). This study analyzed the first 17 years of collected data, 1992-2008, because this is when the sea turtle management and conservation project in Añasco, Puerto Rico was officially managed by the Department of Natural and Environmental Resources (DNER). By looking into the temporal trends in the hatching activity of leatherbacks, the continuous conservation efforts by the government and community can be seen positively impacting nesting activity. The historical data also include some reports on stranding, beach erosion, pollution, poaching, amongst other factors that helped determine how the species was affected over time.

The current study aimed to broadly investigate sea turtle populations in Puerto Rico. More specifically, nesting activity (as measured by nesting events and hatching events) over time was examined to determine whether protections provided to sea turtles were resulting in an increase in population. The analysis from this study can be used to compare the data collected from this location in recent years, thus building a broader picture of leatherback nesting activity. The analysis can be used to compare populations nesting at other locations in Puerto Rico during the same time frame. If it is found that on El Balneario, the population is not increasing, as compared to other areas of Puerto Rico, it could mean that the conservation efforts in Añasco are failing. If they are both decreasing, then the conservation efforts in Puerto Rico would be failing. However, this could also mean that global or regional conservation efforts overall are not working.

MATERIALS AND METHODS

Study area

El Balneario of Añasco is a beach with active sea turtle nesting on the west coast of the main island of Puerto Rico. It is also the largest study area. It has a length of 4,618 meters (2.87 miles), and it is located at the coordinates 18.28584 North and -67.19225 West (CCRC 2014). The coast of

Añasco consists of sandy-type beach with several ravines that disembogue at the sea. There are a total of four streams; Quebrada Caguabo, Quebrada Icacos, Quebrada Justo and Caño La Puente. Caño La Puente generates a significant flow of water into the sea. The vegetation in this area at the time of the study was mostly composed of Portia trees (*Thespesia populnea*), tropical-almonds (*Terminalia cattapa*), creeping vines (*Canavalia maritima, Ipomea stolonifera*, *Ipomoeae pes-caprae*), palm trees (*Cocos nucifera*), coastal dropseed (*Sporolobus virginicus*), and buffelgrass (*Cenchrus ciliaris*) (DNER 1992-2008).

Data collection

The largest species of sea turtle is the leatherback sea turtle (Van Buskirk and Crowder 1994). Nesting leatherbacks in the Caribbean and Atlantic have a curved carapace length of approximately 150 cm – 160 cm (Van Buskirk and Crowder 1994, Eckert et al. 2012). Females of the nesting population in the Western Atlantic range from 327 to 392 kg (Eckert et al. 1989a). In 1994 a dead adult female leatherback sea turtle was found in the town of Barceloneta, Puerto Rico (DNER 1992-2008). The female weighed 346 kg. To the best of the author's knowledge, no published research has investigated the size and weight of leatherback sea turtles in Puerto Rico. The size and weight of this species help surveyors identify nesting activity much easier than with other smaller sea turtles because this species tends to leave the most enduring and identifiable tracks on the beach (Hall 1998). The nest is also easy to identify because Leatherback females lay larger eggs compared to other sea turtles (Van Buskirk and Crowder 1994).

Nesting and hatching activity abundance and hatching success was used to evaluate the effectiveness of nest protection as a conservation tool. This is a similar approach to what Dutton et al. (2005) did, but they used “nester abundance” instead of hatching activity abundance because they tagged individuals in order to count more accurately. In this study, there are not enough individual counts of tagged adult sea turtles to be able to use the same approach as Dutton et al. (2005). A hatching event is a case in which a nest was found to have at least one hatchling emerge from the nest on its own accord or with assistance from a volunteer. How hatching events were documented is explained later in this section.

The data were compiled from a beach in Añasco that was monitored for sea turtle nesting activity by the DNER day and night, 2 to 7 days per week. Because the weekly monitoring by the DNER was not consistent throughout the years, the total numbers for each year are considered as minimums. The different types of reports include nesting, adult female metal tag numbers, female length and width measurements, stranding, hatching, the nest hatching inventory, and relocated nests. Staff relocated "doomed" clutches if necessary (Dutton and Whitmore 1983). Nests classified as "doomed" were found in erosion-prone areas or below the high-water mark, where they could be washed away by the tides. These were relocated to an area on the beach with less risk in a hand dug nest with a shape and size that simulated a natural nest. Nesting was confirmed when an adult turtle was visualized or when there was evidence of hatching. Nests were counted either by observing hatchlings emerging from the sand finding the nest by following the tracks of the hatchlings up to where they emerged, or by using a thin rod to detect hollow spaces and digging in the sand until finding the eggs. When the nest was found with eggs in it or with hatched eggs, it was counted as a confirmed nest or as a successful hatching. Hatching was inferred by seeing the hatchlings or indirectly by finding hatched eggs in a nest. Nests that did not show hatching activity after being given approximately 70 days, were sought using a thin rod. Hatching nests were dug the same day or the next day to inventory and prevent deaths of hatchlings that are trapped between roots, a layer of compact sand or because they are weak. If no eggs were found, it is considered to be a false nest. A false nest is the activity of a turtle coming out of the water, creating a nesting area without laying eggs, and returning to the water.

The nesting areas were not commonly identified with flagging tape or other identification recognized by the public to avoid potential egg thefts. The coastline of Añasco was demarcated based on points every twenty meters from Barrio Caguabo of Añasco to Añasco Abajo with the delimitation of the Rio Grande de Añasco, obtaining 1 - 250 points or marks from north to south. The demarcation of the littoral was done using a phosphorescent spray or reflective tape in trees or structures near the nesting area to use as reference in finding nests (DNER 1992-2008). The surveyors kept track of where the nests were relative to the markers. In the later years, they noted the location of the nest using GPS coordinates. This was done to be able to find where the nests were to determine whether they hatched. Surveyors created a map to go back to the nesting activity location. If they did not witness the hatching, they used the map to find it, dig it up, and see if hatched eggs were present. On some occasions, they witnessed the hatching of nests they did not previously know existed.

Due to a variety of limitations with the data collected and the methods used in its collection, the way these data were analyzed is limited. There was a control for variations in the frequency of data collection and the methods of that data collection that varied over time. For example, a raw analysis of the number of hatchlings would not be a reliable variable because the frequency of observations was not consistent over time. This could cause fluctuations in the total number of hatchlings as a result of increased observation in certain years and decreased observation in other years.

A more effective way of analyzing the data was to simply consider the number of nests. For example, in years when the frequency of observations was reduced to fewer times per week, it is still reasonable to expect that any turtle nest would have been observed over the typical period that a nest is present on the beach prior to hatching. That data was considered a reliable source for analysis. Additionally, hatching success and emergence success was analyzed by evaluating the relationship between successful hatchlings from any given nest, eggs that were not viable, and hatchlings that died in the nest. A number of statistical models were employed to perform these analyses. They are discussed in further detail below. The results of these analysis were compared with other studies conducted around the coast of Puerto Rico and a separate smaller Puerto Rican island municipality named Culebra. Culebra, like other beaches in mainland Puerto Rico, has yielded published studies related to nesting population of leatherback sea turtles (Tucker and Frazer 1991, Tucker 1989, Rivera-Muniz et al. 2000, Dutton et al. 2005, Rosado-Rodríguez and Maldonado-Ramírez 2016).

Statistical treatment

The hatching and nesting events in the years 1992-2008 was separated into two bins. Bin 1 compiles event samples collected from 1992-1997 and bin 2 collects event samples measurements collected from 2004-2008 in the Añasco region area. However, all data from 1996 was removed from the first bin for two reasons: first, removing 1996 from the analyses ensured that the number of years per bin were equal (five years per bin). Second, 1996 was removedbecause that was the only year Añasco was not the primary beach monitored for sea turtles. This means that El Balneario was reported as not being monitored the same as the years before and after, and another beach was the main focus of sea turtle nest monitoring during 1996.

Nesting events

As previously discussed, there is a broad collection of data regarding nesting and hatching activity. The number of nests found was recorded, regardless of whether they hatched or not, to see how many of the sea turtle activities that were marked as being nests actually hatched. Additionally, the characteristics of the nesting female leatherback sea turtles such as the average Curved Carapace Length (CCL) from notch to tip was also evaluated. Shapiro-Wilk normality tests were used to see which parametric tests could be used evaluate the nesting event variables examined. The Shapiro-Wilk normality test helps determine if the variables are normally distributed. Due to the variation in observed and measured females across years, the difference in leatherback nesting and hatching events between years was evaluated using Kruskal-Wallis tests. Years and nesting events are not continuous, therefore this nonparametric version of a one-way ANOVA test was the appropriate statistical test. The sample used was nest and hatching events from 1992 to 2008 of El Balneario to see if there was a statistically significant difference in the number of leatherback sea turtle nest events between years on the Añasco beach. Lastly, the nonparametric Mann–Whitney U test was used to evaluate whether a statistically significant difference in nesting events between bins was apparent.

Hatching events

A single hatching event had a number of different variables documented (e.g., hatched eggs, non-hatched eggs, yolked eggs, yolkless eggs, eggs with embryo). Within those variables, the term status of the embryo was separated into three categories: small, mid, and full term. Small term being the embryo looking mostly pink, medium are black but with the egg yolk still being a big part of the turtle and the term where it is first noticeable it is a sea turtle embryo, and the full term is when the egg yolk mass is closing up into its belly and the turtle looks fully developed and ready to head out of the egg. These variables were also evaluated through means and standard deviation. The test used to see if the hatching event variables analyzed follow a normal distribution was the Shapiro-Wilk normality test. Kruskal-Wallis test was used to investigate whether there was a difference in the number of leatherback sea turtle nesting and hatching events across years on the beach. Following this, a Mann–Whitney U test was used to see if there was a significant difference in the number of nesting events between bin 1 and bin 2.

Hatching and emergence success

Egg clutch, hatching and emergence success was calculated in each individual hatching event, the average for each event calculated separately, and compared between bin years (bin 1 1992-1997, bin 2 2004-2008). Hatching success was calculated as the number of hatched eggs divided by the total number of yolked eggs in a clutch multiplied by one hundred. Emergence success was calculated as: [(number of hatched shells - number of dead hatchlings in the nest)/total number of yolked eggs] multiplied by one hundred. Emergence success is an estimate of the percentage of sea turtles that made it out of the nest and onto the surface of the beach, accounting for some situations where hatchlings die in the nest and never make it out (e.g., due to sand becoming compacted). Shapiro-Wilk normality test was used to evaluate if these variables were normally distributed. A Spearman rank correlation was used to determine if there is a relationship between hatching and emergence success. Expecting the data not to be normally distributed, Wilcoxon Rank tests were used to calculate statistically significant difference of averages between bins 1 and 2 for hatching success and emergence success.

Precipitation and temperature data were referenced because it can influence hatching and emergence success results (Kraemer and Bell 1980, Ackerman 1997, Houghton et al. 2007, Tomillo et al. 2009, Swiggs et al. 2018). The most reliable weather data were from a weather station in a city on the east side of the island at San Juan Luis Muñoz Marín International Airport. These data was downloaded from the National Oceanic and Atmospheric Administration's (NOAA) National Centers for Environmental Information.

Disturbances to leatherback sea turtles

The evaluation of the effects anthropogenic factors have on hatching and emergence success was done by taking note of each situation mentioned in field notes that was affecting nests and its contents (i.e. eggs, hatchlings). Disturbances were separated in three main categories: poaching, light pollution, and compacted sand. Disturbance events that are categorized as poaching can include the killing of leatherback female nesters and the removal of eggs from nests. Light pollution is counted when field notes point out a light source that directly shines on the nesting area and staff believes is affecting sea turtles. It is also counted every time there is a triggered disorientation due to these light sources that avoided some hatchlings to reach the water. Lastly, compacted sand was commonly noted when multiple horse tracks, 4x4 vehicles tracks and that of any other heavy machinery were seen over nesting area. Field notes mentioned compacted sand trapped emerging hatchlings inside the nest and eventually cause their death. The total disturbances in their separate categories were compared between bin 1 and bin 2 and observed overall (1992-2008) to see if these types of anthropogenic activity could be changing over time and affecting sea turtle nests. To address whether there was a correlation between anthropogenic disturbances and the nest count of leatherback sea turtles per year on the Añasco beach, the Shapiro-Wilk normality test was used to determine whether or not the disturbance variables are normally distributed. A Spearman correlation was used to compare a variable called disturbances and the hatching and nest count across years. The disturbance variable included the number of anthropogenic disturbance reports categorized by the most commonly observed (i.e. poaching, artificial light pollution, compacted sand.). The Disturbance variable was categorized as variable 1 = poaching, 2 = light pollution and 3 = compacted sand. A chi-square test of association was also conducted to determine whether the proportion of successful hatching events differed across different disturbances (none, poaching, light pollution, and compacted sand). A comparison was made of the percentage of disturbances recorded within each bin, as well as percentage comparisons between bin 1 and bin 2 separately.

RESULTS

Curved Carapace Length

A sample of 425 leatherback sea turtle nesting and hatching events were analyzed in bin 1 from 1992 – 1997 and bin 2 from 2004 – 2008 (120 and 304, respectively) in the Añasco region. Normal measurements of CCL from notch to tip in Leatherback turtles were averaged from a total of 145 measurements, with a total of 37 measurements in bin 1 and a total of 108 measurements in bin 2. The averages are represented on Figure 1. with 1.6 meters ±SD 0.11 and 1.49 ±SD 0.16 respectively for bin 1 and bin 2.

Nesting events

Nesting events were recorded as early as February 13 and as late as August 4 from 1992 to 2008. February 13 being the earliest for both bin 1 (1992-1997) and February 14 for bin 2 (2004-2008), and July 8 being the latest for bin 1 and August 4 for bin 2. The observed number of leatherback female nesting turtles has ranged annually between 1 to 18 from 1992 to 2008. The nesting events varied significantly between years [Kruskal-Wallis H (90) = 109.69, P = 0.08]. The largest number of nesting events of leatherback turtles in the Añasco region is seen in 2006 (Figure

2). Also, the years that follow 2006 show an increase in the number of nesting events compared to the previous years, with a count of 81 or higher for the most recent years. Additionally, trends in the nesting distribution of leatherback turtles from 1997 to 2005 were almost even. Leaving the lowest of nest counts from year 1992 to 1993. Results showed nesting events in bin 2 significantly higher than the nesting events in bin 1 (Mann–Whitney $U = 1$, $P < 0.05$).

Hatching events

Hatching events were recorded to start from April and end in September from 1992 to 2008. May being the earliest for bin 1, April for bin 2, and September the latest for both bins. The observed number of hatching events increased annually from 3 in earlier years to 59 throughout 2008 (Figure 3). The hatching events were significantly different between years [Kruskal-Wallis H (11) = 20.055, P = 0.04]. The highest number of hatching events from Leatherback turtles in the Añasco region is seen in 2006. Years from 2006 and above show an increase in the number of hatching events compared to all other years with a count of 81 or higher (Figure 3). A trend in event distribution can be seen across years 1995 to 2005. However, the trend from previous years can be seen rapidly changing in 2006. Hatching events were significantly higher in bin 2 than bin 1 (Mann–Whitney U = 2, $p < 0.05$).

In Table 1, frequencies and means can be seen of the hatching activity in Añasco, Puerto Rico. Within each nest, the egg contents within a clutch were counted and categorized as hatched, unhatched (with and without dead embryos), rotten (visible bacteria and fungi present), and embryo term stages (small, mid, and full). The data demonstrate the count of hatched eggs within clutches in bin 1 as higher than bin 2 with a mean of 72.49 ± SD 19.26. On the other hand, the average percentage of rotten eggs was higher in bin 1.

Hatching and emergence success

Shapiro-Wilk normality test results demonstrated that emergence and hatching are not normally distributed (alpha level of 0.05, p value < 0.05). For this reason, a Wilcoxon Rank test was used to calculate statistical significance difference of the average because hatching and emergence success are strongly correlated (as seen in Figure 4) and Wilcoxon can be used for dependent samples. Figure 4 also shows a number of high hatching success events with zero emergence success (bottom-right corner). Results showed that hatching success between bin 1 and bin 2 are significantly different (alpha – 0.05 p – value <0.05). The 1 side Wilcoxon Rank test showed that the average mean in hatching success within bin 1 is statistically higher than in bin 2 (see Table 2). The emergence success rate displayed the same results with a p value < 0.05 (Table 2).

Precipitation and Temperature

The amount of times precipitation and rain was mentioned throughout the years in bin 1 (n=9) were higher than bin 2 (n=4). Additionally, reports note two hurricanes affected nests in 1995 and one in 1997. Although field notes give little information to come into solid conclusions, one could suspect that there was potentially more precipitation during bin 1 compared to bin 2. These data showed a yearly average precipitation of 47.65 for all of the years averaged together in bin 1 and 63.45 inches during bin 2 (NOAA 1992-2008). The yearly average temperature was 81.02°F for all of the years averaged together in bin 1 and 80.52°F in bin 2 (NOAA 1992-2008).

Disturbances to leatherback sea turtles

For other variables like anthropogenic disturbance in the leatherback population from years 1992 to 2008, Spearman correlation was used. Because of the missing values in the disturbance variable, a clear correlation could not be seen between hatching event and disturbances (Figure 5) and the same result came with nesting event and disturbances (R= -0.14, p value = 0.048). However, the overall disturbances seen from year 1992-2008 in the leatherback turtle population in the Añasco region could still be visualized through Figure 6. The chi-square test of association was statistically significant, $\chi 2 = 10.54$, df = 3, p = 0.014, implying that the proportions of successful hatching events differ between disturbance events. The contingency table (Table 3) and the bar chart (Figure 7) show that light pollution is associated with a visibly lower hatching success rate than no disturbance, poaching, and compacted sand.

As seen in Figure 6, light pollution was more common following compacted sand and lastly poaching. When comparing difference between years in bin 1 and bin 2 (see Figure 6B and 6C) light pollution was more common in earlier years (bin 1). On the other hand, compacted sand can be seen being the most common disturbance in bin 2 following light pollution. For both bins, poaching was the least common disturbance on the nesting sites.

DISCUSSION

The level of nesting and hatching activity of *D. coriacea* increased from bin 1 to bin 2, potentially indicating an increase in population. This may indicate that conservation efforts have been successful, as opposed to observing a stagnant or decreasing level of nesting and hatching activity—which could indicate that the population is not growing or in decline. Conservation efforts such as community involvement in nesting beaches and species protection may have contributed to the increase in nesting and hatching activity. Remigrant leatherback sea turtles could have contributed to the increase of nesting and hatching activity at the study site by specifically selecting to nest on El Balneario as opposed to neighboring beaches. Annual reproductive success decreased from bin 1 to bin 2, potentially a result of factors in the nest environment or various anthropogenic factors. Temperature and precipitation can either create a productive nest environment or on the contrary one filled with bacteria and fungi. Anthropogenic factors such as light pollution, poaching and compacted sand may have affected nesting activity, hatching activity and reproductive success. Studies have indicated that sea turtle populations decrease in areas with less protection and increase in areas with greater levels of protection (Eckert 2001, Dutton et al. 2005).

Curved Carapace Length

Normal measurements of Curved Carapace Length from notch to tip in Leatherback turtles is reported to be 1.50 m to 1.60 m in other studies (Van Buskirk and Crowder 1994, Eckert et al. 2012), including at a separate smaller Puerto Rican island municipality named Culebra where they CCL was reported to be 1.55 m (Tucker and Frazer 1991). Consistency of CCL leatherback sizing was observed in Añasco Leatherback turtles (Figure 1). This knowledge provides baseline information on the morphology of leatherback sea turtles in Puerto Rico.

Nesting events

Nesting numbers increased as the years progressed, resulting in less recorded nests in bin 1 and more during bin 2 (Figure 2). This is consistent with what Horta-Abraham et al. (2003) found in their leatherback nesting study done on the northeast of Puerto Rico. During 17 years of their study that ranged 1986-2002, Horta-Abraham et al. (2003) saw an increase in nesting, hatching, nesting female, and remigrant leatherback sea turtle activity. Rivera-Muniz et al. (2000) conducted a study from 1997 to 1999 in southeastern Puerto Rico where their number of nests of *D. coriacea* per year ranged from 53 to 71. However, as years progressed, the nesting activity numbers decreased, 1997 having the highest nest count of 71 and 1999 the lowest at 53 (Rivera-Muniz et al. 2000). In our study, the year 2006 has the highest nest count and also the highest observed female nesters (n=18). The high values in nest count in 2006 could be explained due to having more female nesters observed during that year. Leatherback turtles remigrate at 2-3+ year intervals (Eckert 2001). The coincidence of these 2-3+ year intervals may be one reason that 2006 has the highest nest count and overserved female nesters. It is also possible that there is a higher population of female nesters in recent years due to the high hatchling production found in the earlier years of this study. The proposed average age of maturity for leatherback turtles is 9-15 years (Zug and Parham 1996). Therefore, this could be consistent with the increase in adult nester numbers in recent years. Resiliency to dangers at sea and on land while nesting can also contribute to nesting activity increasing in the area in recent years. Reports on leatherback nesters showed the return of multiple individuals regardless of exhibiting visible wounds which were healing (DNER 1992-2008). This increase in the recordings of nesting activity could also be explained due to an increase in volunteer and staff work hours as part of the program development over the years. I was unable to confirm whether there was an increase in volunteer and staff work hours over the years. Community involvement in the protection and reporting of nesting and hatching activity to the DNER may also have influenced the increase of nesting activity recorded by staff. More about community involvement and other conservation efforts will be explained in the Disturbances to leatherback sea turtles section below.

The lowest nest count values were recorded during 1993. There are several possible reasons for this: First, DNER staff reported in 1993 that residents who live near the beach at El Mani, Mayagüez commented that a few people had threatened to kill any turtle that would come out of the water to nest. This beach in Mayagüez is immediately south of the beach in this study, which makes it possible that any poaching on that beach may affect sea turtle populations in Añasco. Leatherbacks may travel to nearby beaches or even other islands to nest as seen in Culebra, Puerto Rico (Eckert et al. 1989b). There have been documented cases of inter-nesting for some of Culebra's female *D. coriacea* on other beaches within the US Virgin Islands as well as Culebra and mainland Puerto Rico (Eckert et al. 1989b, Horta-Abraham et al. 2003). Second, an unauthorized deforestation of part of the nesting beach in Añasco was reported to potentially having a negative effect to nesting by exposing the nesting area to artificial lighting. Artificial lighting was the most mentioned disturbance during bin 1 and serves as a deterrent to oviposition and disorients sea turtles (Hall 1997, Silowsky 2018). Third, reports said that a lot of debris was found on the beach, and they recommended beach cleanups to take place in order to better the access to nesting turtles. If this suggestion was taken into consideration, it could have increased nesting numbers in later years. Lastly, illegal extractions of sand were reported to have increased during 1993, which may have caused the loss of new nests or erased turtle tracks on sand to identify new nests (although t. he reports did not mention the extent of the illegal extractions).

Hatching events

Hatching events also increased as years progressed, especially in 2006. The reasons for this can be similar to the reasons for increase in nesting events which include more female nesters due to remigration and higher hatchling production (hatching and emergence success) found in bin 1 that reached reproductive maturity. Increase in monitoring hours as well as higher community involvement in the protection of the nesting area as well as the turtles themselves are other factors that can also contribute to this increase in hatched nests found. Lastly, the survivability of *D. coriacea* in the wild can also influence the higher numbers of hatched nests found.

Hatching and emerging success

The association between hatching success and emergence success was also investigated. As figure 4 demonstrated, the variables are strongly correlated, although a number of nests did have high hatching success but zero emergence success. Both hatching and emergence success decreased as years progressed. In a study conducted in southeastern Puerto Rico, Rivera-Muniz et al. (2000) saw a decrease in nesting activity from 1997 to 1999 but an increase in hatching and emergence success. In the current study, mean results for egg clutch revealed more hatched eggs in bin 1 (1992-1997) compared to bin 2 (2004-2008), and more rotten eggs in bin 1 compared to bin 2.

Differences between bins could be due to precipitation differences throughout the years. Precipitation and rain were mentioned more in bin 1 than bin 2, however NOAA weather data from San Juan Luis Muñoz Marín International Airport showed greater precipitation during bin 2. It should be noted that the spatial distribution of precipitation varies from the east to the south side of the island (Vélez et al. 2019). The NOAA precipitation data was the opposite of what was expected for precipitation differences between bins based on the notes from the study. The average temperature for all of the years in bin 1 at the San Juan Luis Muñoz Marín International Airport was higher than the average for bin 2.

During development, sea turtle eggs can tolerate a range of temperatures that falls between 77–80.6 and 91.4–95°F (Ackerman 1997). High temperatures have a negative effect on eggs and hatchlings during incubation period and emergence, hence reducing the production of hatchlings and their emergence from the nest (Tomillo et al. 2009). Lower temperatures can result in both higher emergence and hatching success, and precipitation can have a cooling effect in leatherback nests (Houghton et al. 2007, Tomillo et al. 2009). However, too much precipitation can drown the eggs if the sand does not drain well (Kraemer and Bell 1980). If precipitation in bin 2 were to be higher to the point of flooding the nests at some point during the embryo development, this may be the reason why there was lower hatching success during the last years of the study. Nevertheless, some water draining from the surface to the nest cavity can help emergence success by reducing hatchling dehydration as they ascend through the sand column (Swiggs et al. 2018).

The higher number in rotten eggs found within bin 1 may also be explained due to the fungi *Fusarium solani* that is found to affect leatherback sea turtle nests in Añasco (Rosado-Rodríguez and Maldonado-Ramirez 2016). Study samples from Rosado-Rodríguez and Maldonado-Ramirez (2016) were taken during 2008 and 2009 leatherback nesting seasons. Rosado-Rodríguez and Maldonado-Ramirez (2016) said *F. solani* can negatively affect embryo development and contribute to egg failure during incubation. *F. solani* was found to infect the interior and exterior surfaces of eggs. It has also been found on the skin and carapace of dead leatherback hatchlings (Miller et al. 2009). The fungi proliferate in high humidity and temperatures. Therefore, with bin 1 mentioning precipitation more than bin 2, it could help explain why the average number of rotten eggs per hatched nest were higher during the earlier years. However, given the discrepancy between the notes on precipitation and the NOAA data, it is also possible that during the first years the people recording the data might have been more observant when taking notes on precipitation and hurricanes affecting nests compared to the later years.

Additionally, bacteria found in sand and transferred by female nesters can also affect the nest clutch hatching success (Wyneken et al. 1988). *Serratia* is a genus of a bacteria found in nonviable eggs of the loggerhead sea turtle (*Caretta caretta*) in the Wyneken et al. (1988) study and it is suspected to negatively affect the health of embryos (König et al. 1987, Gordon et al. 1998). The widespread opportunistic pathogen *Serratia marcescens* can cause disease in humans such as pneumonia and urinary tract infection (Maki et al. 1973, Lyerly and Kreger 1983). Specifically, *S. marcescens* has been found in green turtles (*Chelonia mydas*) with disseminated infection and septic aortic thrombosis in locations such as the spleen, cerebrospinal fluid, and pericardial fluid (Gordon et al., 1998). *S. marcescens* can unfavorably affect sea turtle embryo development due to its hemolytic properties (König et al., 1987). In a study by Smith et al. (2007), *S. marcescens* was found in a single egg albumen sample at our study site in Añasco, Puerto Rico, which could indicate that it could have been a contaminant to leatherback sea turtle eggs during bin 2. More studies should be done on bacteria presence at the beach near contaminants which may be causing the bacterial presence.

Lastly, other factors that contributed to the loss of nests included predation by dogs and ants, and nests falling into Caño La Puente when the river mouth opened. Being close to the high-water mark can also contribute to a low hatching success by flooding or nests being washed away by the high tides (Tomillo et al. 2009).

The monitoring of nest location in regards to the high tide, bacteria presence in nests, temperature, humidity, and sand characteristics within nests should be considered in future studies at the beach in Añasco due to being factors that contribute to the success of clutches (Kraemer and Bell 1980, Gordon et al., 1998, Wyneken et al. 1988, Ackerman 1997, Houghton et al. 2007, Smith et al. 2007, Tomillo et al. 2009).

Disturbances to leatherback sea turtles and conservation efforts

Anthropogenic factors play a role in both sea turtle recovery as well as their endangerment (Bjorndal and Jackson 2003, NALWG 2018). In this study, the disturbance variables created (poaching, light pollution, and compacted sand) did not show any correlation between years, but hatching success was visibly lower if the nest area was affected by light pollution (see Table 3 and Figure 7) An analysis of these variables can help better understand the effects of certain conservation efforts implemented by DNER staff, volunteers, the local community and changes in public policy and its application. In bin 1 there were more reports that pointed towards the presence of light pollution and in bin 2 there were more reports mentioning compacted sand. There were low numbers of nesting activity during bin 1 which could be explained by the light pollution serving as a deterrent to oviposition and disorienting nesting sea turtles (Hall 1997, Silowsky 2018), potentially encouraging them to nest in a neighboring beach (Eckert et al. 1989b, Horta-Abraham et al. 2003). Compacted sand created by human use can affect hatching and emergence success numbers and could be a reason for some of the low percentages found during bin 2 (Mann 1977, Kudo et al. 2003). Mann (1977) found that emergence success can be negatively affected by vehicles driving over nests and can even kill hatchlings. Due to this fact, compacted sand is something that could have prevented the hatched nests from emerging in this study, as seen in the bottom-right corner of Figure 4. A common cause for compacted sand was horse riding on the sand and off-road vehicles driving over nests, the latter one being the most common. Surveyors reported finding hatchlings trapped inside nests that were driven over by vehicles. Other causes for compacted sand included the use of machinery to open the Caño La Puente river mouth. The DNER reported that machinery is brought in for the purpose of helping water drain so the farms up the river do not flood.

Poaching was the least mentioned in the reports for the study site. However, it is possible that poaching events directed to nesting females committed on adjacent beaches can affect the nesting female population at the study site. This is because nesting females found at the Añasco study site have been seen on other nearby beaches. There was a leatherback sea turtle beheaded in 1992 in

Mayagüez, the town adjacent to Añasco. In 2007, there was another leatherback sea turtle found dismembered on the southeastern side of the island at California beach in Maunabo, Puerto Rico. The DNER offered to pay up to $10,000 to anyone who helped find the person(s) responsible for this event. This economic reward is one of the tools the DNER can use to prevent poaching and promote conservation. Other anthropogenic factors found at the study site included fishing nets being left on the sand overnight which surveyors mentioned can cause entanglements (DNER 1992-2008).

High numbers of nesting activity shown as years progress can also be explained due to the DNER conservation efforts and community involvement (DNER 1992-2008). Beginning in 2001, reports on effective conservation efforts and community involvement were written with a sense of pride and continued through 2008. In 2001, a report stated that the number of poaching of nests as well as the killings of female nesters had been reduced, nesting beaches were being better protected in relation to adjacent anthropogenic development, there was direct volunteer community involvement in monitoring of the beach during day and night patrols, beach cleanups and coastal reforestation were occurring, and local residents were collaborating to reduce artificial illumination towards the beach. Hall (1998) also reported that individuals, families, and communities have developed a positive attitude towards sea turtles in Añasco since educational efforts began in 1992. Hall even reported that poachers in Añasco seemed to abandon their practice after seeing the community increasingly interested in being involved in the caring of “their” sea turtles (Hall 1998).

In conclusion, this study provided baseline information for nesting, hatching and emergence success data, and conservation efforts and management practices at the El Balneario beach in Añasco that can be used as references for future studies regarding these practices in Puerto

Rico and elsewhere. There was an increase of nesting activity and hatching activity that can likely be allocated to successful conservation efforts by DNER staff, volunteers, and the community due to educational efforts and increased stewardship. There is still much more to be learned from sea turtle activities on this beach. For example, an analysis of clutch distance in regard to the high tide and its success should be explored in the future. This analysis can help evaluate if clutches at this beach that are closest to the high tide have a higher hatching and emergence success than some farther from it, as seen in Tomillo et al. (2009). Additionally, other topics of research in the future could evaluate the theories presented in this study regarding the relationship between hatching and emergence success with bacteria and fungi presence, sand characteristics, and temperature and humidity.

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