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A Correlation Study of the Nest Incubation Period and Hatchling Development and Success Rate for Olive Ridley Sea Turtles (Lepidocheyls olivacea) Turtle Conservation: Playa Junquillal, Costa Rica

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Introduction

Listed as vulnerable by the International Union for the Conservation of Nature (IUCN) the Olive Ridley sea turtle contributes to the stability of an ecosystem by transferring nutrients and energy to both the terrestrial and oceanic environment [1]. Costa Rica holds some of the most prominent nesting beaches for Olive Ridley sea turtles in the World. With seasonal and mass nesting events, such as *arribadas*[1], Costa Rica is immensely important to the life cycle of the Olive Ridley sea turtle. The protection of the Olive Ridley is necessary, as their population has decreased significantly on a yearly basis. Nests left *in situ* have a hatching success rate of 33% [2]. It is estimated that human activities destroy 2% of *in situ* nests; animal predation disturbs roughly 25% and beach erosion around 16% [2].

Collection and relocation of surveyed nests to monitored hatcheries increases the success rate significantly [2]. Realistically, nest and hatchling protection against land-based threats such as poaching, predation and beach erosion is a practical and accessible conservation measure.

After eliminating these hazards to the nests, hatcheries face environmental threats including microbial growth surrounding the nest as well as in the eggs [3]. Microbes can damage or kill developing embryos. Removing old nest sand in hatcheries allows for more optimal conditions. Fresh sand improves the balance of nutrients, biotic elements, abiotic elements and physical conditions (e.g. nest size, depth, and compactness of sand) of the nest and allows for diffusion of oxygen, carbon dioxide, and water that positively impacts embryonic development [3].

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[1] A Spanish term used by locals to describe the reproductive phenomenon where marine turtles display a synchronized mass nesting behavior.

Facilitated by access to the sea turtle hatchery at the Playa Junquillal field station, Verdiazul, Costa Rica, the purpose of this study was to analyze and compare development and hatching success to incubation time and clutch size for collected and relocated turtle nests.

Design

The parameters of the experiment followed the Playa Junquillal field station protocol and only examined nests that have already hatched. Qualitative data was collected focusing on possible microbial growth in the nest, stage of development for non-hatched eggs, and nest description after hatching (e.g. smell and moisture). Quantitative data used for the correlation study includes number of eggs per clutch transferred to the hatchery, length of incubation (date of oviposition to date of emergence from nest), number of hatchlings that emerged from the nest, number of turtles that hatched but died while emerging from the nest, number of eggs with embryos, number of embryos in each developmental stage, and the number of eggs without embryos. Graph analysis identified the various frequency distributions of the data as well as the correlation coefficients between possible influential variables and success rate.

Methods

Clutch collection is the first step in the conservation effort and in field station studies[2]. Turtle eggs were collected during or immediately after oviposition from the adult Olive Ridley nests. Adult turtles come ashore to nest at night during the end of low tide and the transition to high tide[2]. Night patrols were thus scheduled based on the times of high and low tides. During the patrols volunteers and staff walked the Playa Junquillal, Honda and Estero beaches, roughly a

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[2] Standard procedures established at the Verdiazul Field Station to harvest marine turtle eggs and relocate them to the hatchery.

three-mile coastline. The patrols watched for evidence of turtle tracks in the sand. Olive Ridley turtles have a distinctive track that is a result of their front forelimb fin[2] If entrance and exit tracks were found the nest was complete and buried indicating that the adult Olive Ridley had returned to the ocean. The process of finding the nest involved following the tracks and estimating a circumference between the entrance and exit tracks where the nest could be located. After identifying the possible location a stick was inserted into the area of the circle until it sank without resistance. Little resistance is a result of the sand not being firmly packed but aerated and indicated the region where the adult buried the nest[2]. Once located, retrieval of the nest involved three people. One individual was responsible for digging up the nest. With gloves the individual dug in a clockwise circle until the eggs were uncovered, approximately a foot and a half to two feet under the surface of the sand. The eggs were then removed from the nest, counted and placed into a bag. The second individual was responsible for holding the bag and counting the retrieved eggs as well. A third individual recorded the data about the nest including location, tides (high or low), beach illumination, the phase of the moon, and any local buildings or manufactured light.

If only entrance tracks were visible the adult turtle was still in the process of making the nest and laying her eggs. The adult Olive Ridley was located by using a red flashlight and following the tracks; two individuals then approached from behind without alerting the turtle to their presence. While the adult turtle continued laying the eggs a single individual wearing gloves caught and removed all the eggs. The second individual held the bag for the eggs to be placed in and counted the number of eggs laid. Once finished with oviposition and egg collection volunteers and staff left the adult Olive Ridley to bury the now empty nest and reenter the ocean. The data from this portion of the study was identified as *Group A* (Appendix A).

The hatchery was a 30’ by 12’ rectangle separated into a 2’ by 2’ square grid yielding 30 locations for relocated nests (Reference Image 1). Each square in the hatchery had a hard plastic net in the sand and on top of the sand to act as a deterrent to predators, such as sand crabs, raccoons or dogs. Each grid location was associated with its row letter, A to F, and its column number, 1 to 15. After retrieval of the nest the collected eggs were moved to the hatchery and their placement was recorded. Appropriate technique to emulate the *in situ* nest environment involved digging the new nest to have the same structure as the *in situ* nest. A column in the sand was created with an 8-inch diameter about a foot and a half below the surface and then expanding the column at the bottom to make it roughly a foot wide. The eggs were recounted and placed into the new nest, then buried by placing the sand back in the hole but not compacting it down similar to how the adult Olive Ridley would cover the nest.

After the Olive Ridley hatchlings emerged from the nests the remaining eggshells and unhatched eggs were removed from the nest and examined by two individuals (Reference Image 2). One individual recorded the data while the second individual performed the examination. The date of oviposition, date of emergence, and examination date were all recorded. In the examination eggs that came from hatched and released turtles were shell fragments. Dead turtles were defined as developed turtles that had broken their shell, fully or partially, but had not survived to be released. Unhatched eggs were opened and embryonic presence and phase of development was analyzed. If the egg was unfertilized, there was only yellow yolk present. Incubation for embryonic development of Olive Ridley sea turtles is roughly 60 days and is categorized into four stages [1]. Embryonic presence was identified and grouped into phases 1, 2, 3, and 4 of development. Phase 1 was a dark region taking up about 25% of the yolk. Phase 2 was a partially developed embryo taking the shape of a small turtle and occupying roughly 50%

of the yolk. Phase 3 was a clear embryo in the form of a hatchling covering 75% of the yolk but did not have eye development. Finally, phase 4 was a fully developed embryo that had not emerged from the shell. The developmental phases were recorded. Along side the developmental phases two more categories were included in the analysis. These were egg infection and vain classification. Infected eggs had a putrid odor and either pink or black growth in the yolk – the bacterial or fungal growth was distributed throughout the yolk and not localized. Vain eggs were empty eggs without yolk, only containing albumin, laid by the adult female as a decoy mechanism to possible predators [6]. Vain eggs were smaller than fertilized eggs and had a fibrous texture. The data collected during the aforementioned procedures comprises *Group B* (Appendix B) and was applied to the analysis of the correlation between incubation period and hatching development and success rate.

The study also includes previous data that was available and collected upon arrival at the field station. This data included original beach location of oviposition and number of eggs and is identified as *Group C* (Appendix C). Original oviposition location was not used in this comparative study. However, it is vital information regarding the release of the hatchlings.

Results

Group A

Group A consists of nests found during nightly patrols that were uncovered and removed from *in situ* positioning and relocated to the hatchery. 13 nests were found and relocated, totaling 1,061 eggs with an average clutch size of 81.6 eggs per clutch. The phase of the moon and the level of the tide were recorded for each nest.

Figure 1a shows that eight of the thirteen nests, roughly 62%, were found during the full moon phase. In contrast only 7.7% of nests were found during a new moon, 7.7% were found during a first quarter phase of the moon and 7.7% were found during a last quarter phase of the moon. Cloud cover with no moon visible, had success with 15% of nests found during this study. No nests were found during half moon phases.

Nightly patrols were scheduled based on the cycle of the tides. Patrols would start at low tide and continue through high tide. From **Figure 1b**, of the 13 nests 11, or 84%, were found during the high tide cycle. Five of the 13 nests were found when the tide was at ¼ of the way to high tide.

Group B

The second phase of the study involved analysis of hatched nests. In total, 28 nests and 2,514 eggs were placed in the hatchery for incubation and development. The average clutch size was 89.8 eggs with an average incubation period of 56.1 days, and the average hatching success of 78.2%.

After hatching, the nest was emptied of any remaining eggs or shells. Remains were divided into two categories, Hatched and Unhatched, with multiple subcategories. A description of each category can be found in the legend of **Table 1**. From the study, 2,042 of the 2,514 eggs (81.2%) successfully hatched. Of the 2,042 hatched eggs, 97.7% of the Olive Ridley hatchlings survived to term and were successfully released (1,994 turtles). 48 of the 2,042-hatched eggs, 2.4%, were fully developed turtles that were not successfully released and died prior to emerging from the nest. The clutch size appeared to have a modest influence on survival and release success. There was a moderate correlation between the clutch size and the number of turtles released after hatching (**Figure 2a**; R= 0.684). A weak positive correlation coefficient was indicative that the percent of live hatchlings released was likely not correlated to the size of the nest (**Figure 2b**; R= 0.156). **Figure 3a** and **b** indicate that the incubation period in association with each nest had no correlation to the number of live hatchlings successfully released (R=0.0882) or to the percentage of live hatchlings that survived (R=0.0077).

Approximately 472 of the 2,514 eggs (18.8%) did not hatch. 322 of the 472 eggs (68.2%) were infertile and therefore not viable for development. The four phases of development referenced in **Table 1** show that roughly 20% of the fertilized eggs had proceeded to stages three and four with a small number of eggs only reaching phases one and two of development. 3.4% of the unhatched eggs displayed characteristics of infection.

Incubation and development is dependent on temperature for many reasons. The presence of microbial species as well as oxygen, water, and CO_2 are also critical factors that can impact development [3]. The average period of incubation from the 28 nests studied was 56.1 days. Analysis of the influence of clutch size on incubation period in **Figure 4** showed that the clutch size did not have a significant correlation (R= 0.061) to incubation period and nests ranging in number from 33 to 152 eggs. All had an incubation period between 48 and 64 days. Two incubation periods had a higher distribution of nests. 9 of the 28 nests were within the 52-54-day incubation period and 7 of the 28 nests were within the 57-58-day incubation period (**Figure 5**).

Combined Data

Data from *Groups A*, *B* and *C* resulted in the analysis of clutch size for 53 nests of the Olive Ridley species. 4,731 eggs were collected and the average clutch size was 89.2 eggs. **Figure 6** shows a frequency distribution of the clutch sizes with the largest number of nests in the range of 91-100 eggs and 101-110 eggs. *Group B* and *C* yielded information for location of original nest position with 93.75% of the nests found on the Playa Junquillal and Estero beaches.

Data

Group A

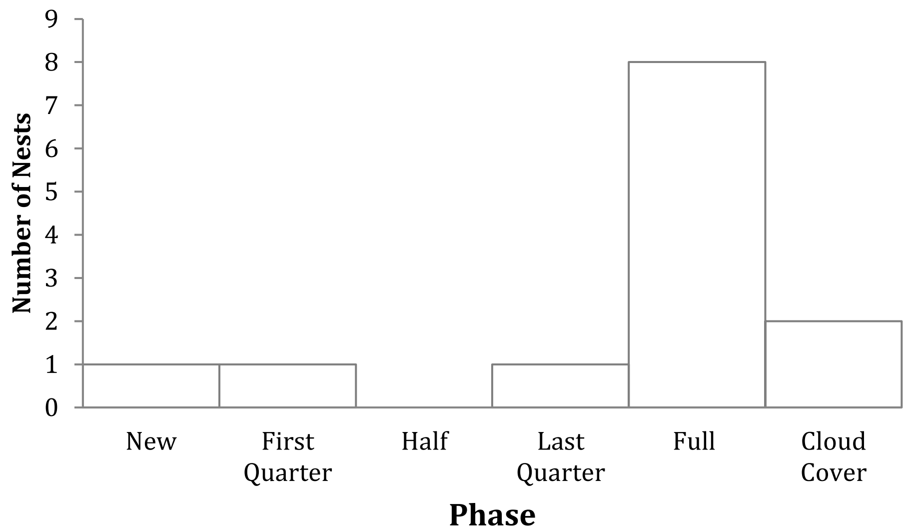

Figure 1a. Representation of 13 nests found along Playa Junquillal, Honda and Estero beaches, during nightly patrols over the course of a 3-month period (June-August) and the phase of the moon when the nests were found. Cloud cover includes various phases where the moon was not visible and there was low visibility on the beach.

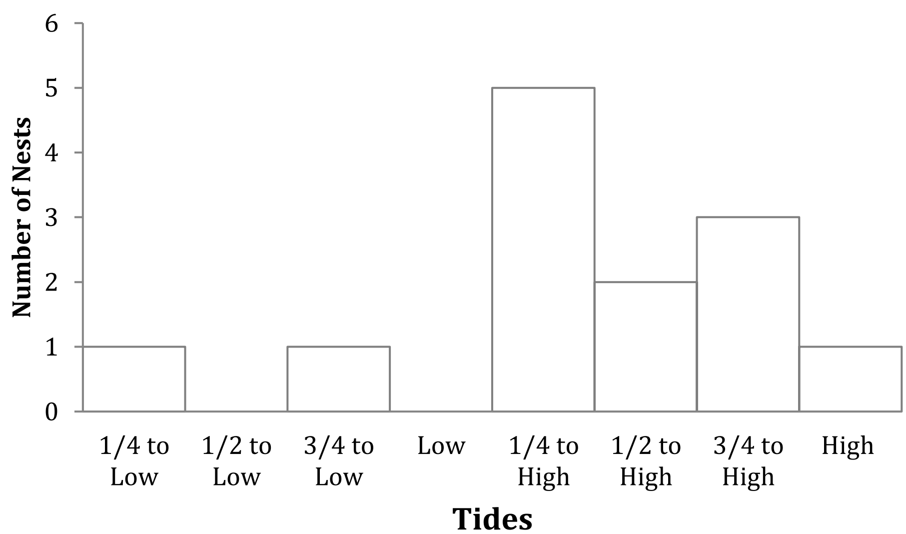

Figure 1b. Representation of 13 nests found along Playa Junquillal, Honda and Estero beaches, during nightly patrols over the course of a 3-month period (June-August) and the ocean tides correlating to the time each nest was found.

Group B

Table 1. Analysis of the hatchery clutches after hatching and release.

Egg Fate	**Number of Eggs**	**Percent of total all eggs**	**Percent of Hatched**
Hatched	2042	81.23%	
Emerged	1994	79.32%	97.65%
Dead in nest	48	1.91%	2.35%
			Percent of Unhatched
Unhatched	472	18.77%	
No Embryo	322	12.81%	68.22%
Phase I	30	1.19%	6.36%
Phase II	18	0.72%	3.81%
Phase III	40	1.59%	8.47%
Phase IV	46	1.83%	9.75%
Infected	16	0.64%	3.39%
Vain Eggs	0	0	0

Table 1. Analysis of the hatchery clutches after hatching and release. The *Hatched* egg categories included - *Emerged*: the number of Olive Ridley turtles that entered the ocean, and *Dead in nest*: developed turtles that had broken their shell, fully or partially, but had not survived to emerge from the nest. The *Unhatched* categories included - *No Embryo:* egg was unfertilized, with only yellow yolk present, *Phase 1*: dark region taking up about 25% of the yolk, *Phase 2*: partially developed embryo taking the shape of a small turtle and occupying roughly 50% of the yolk, *Phase 3*: visible embryo in the form of a hatchling covering 75% of the yolk but did not have eye development, *Phase 4*: fully developed hatchling that had not emerged from the shell, *Infected*: putrid odor and either pink or black bacterial or fungal growth in the yolk –distributed throughout the yolk not localized, and *Vain Eggs*: empty eggs without yolk laid by the adult female as a decoy mechanism to possible predators.

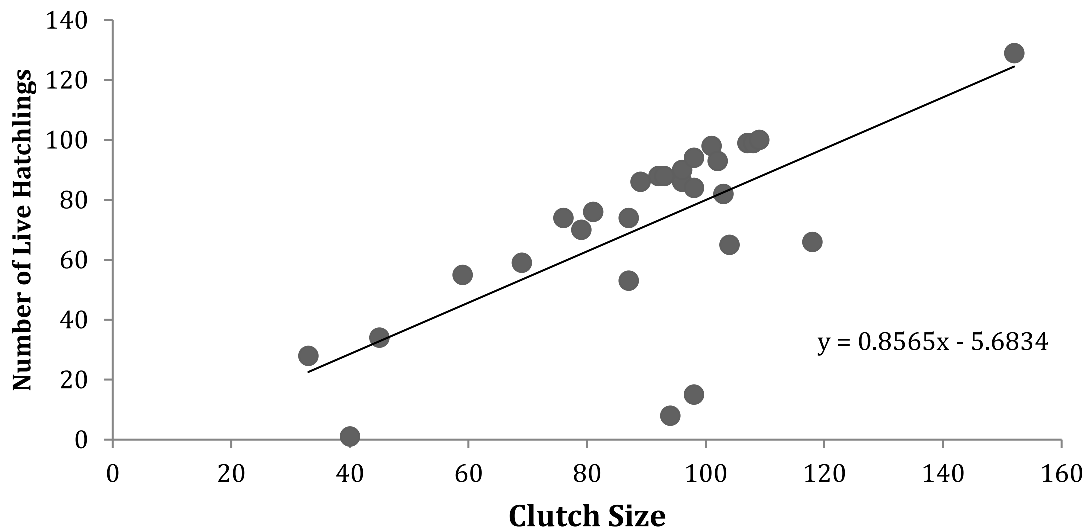

Figure 2a. Correlation analysis between clutch size (number of eggs) of 28 nests and the number of live hatchlings observed upon release. Correlation coefficient: R = 0.684.

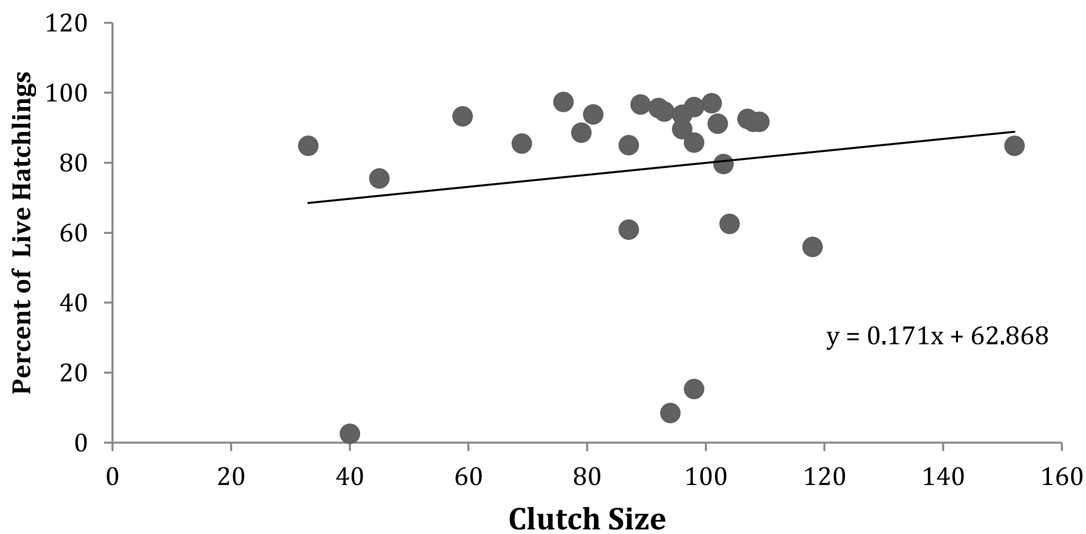

Figure 2b. Correlation analysis comparing the clutch size of 28 nests to percent of live hatchlings released. Percent live hatchlings = (number of living turtles/original clutch size) x 100. R = 0.156.

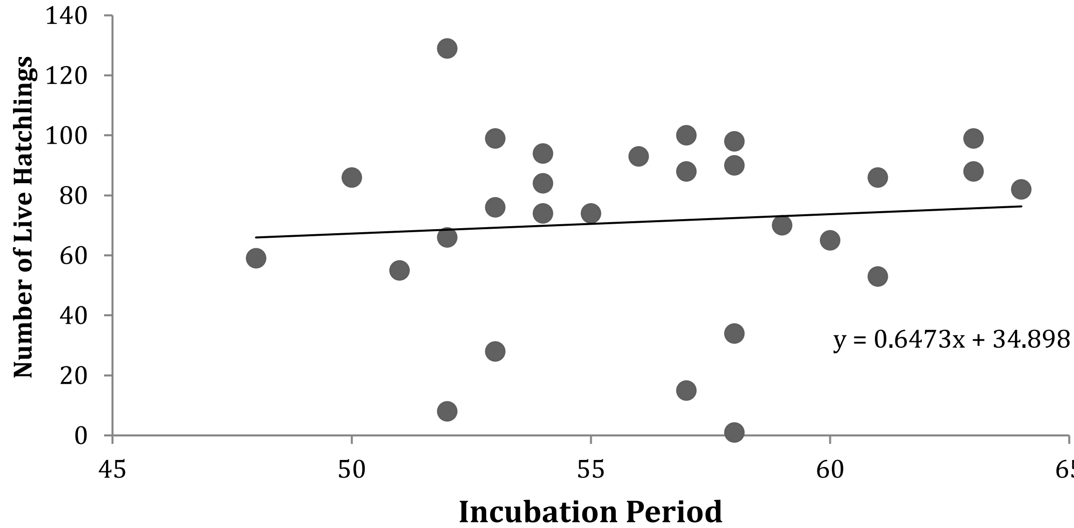

Figure 3a. Correlation comparison between the incubation period of 28 nests and the number of live hatchlings released. R = 0.0882.

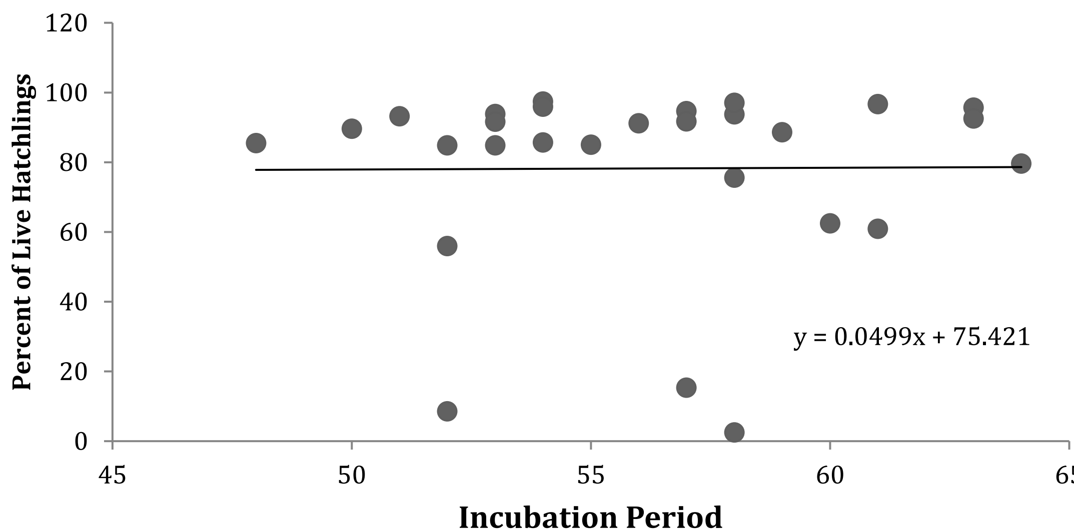

Figure 3b. Observation of the correlation between the incubation period and the percent of live hatchlings released for 28 nests. Percent live hatchling = (number of living turtles/original clutch size) x 100. R = 0.00774.

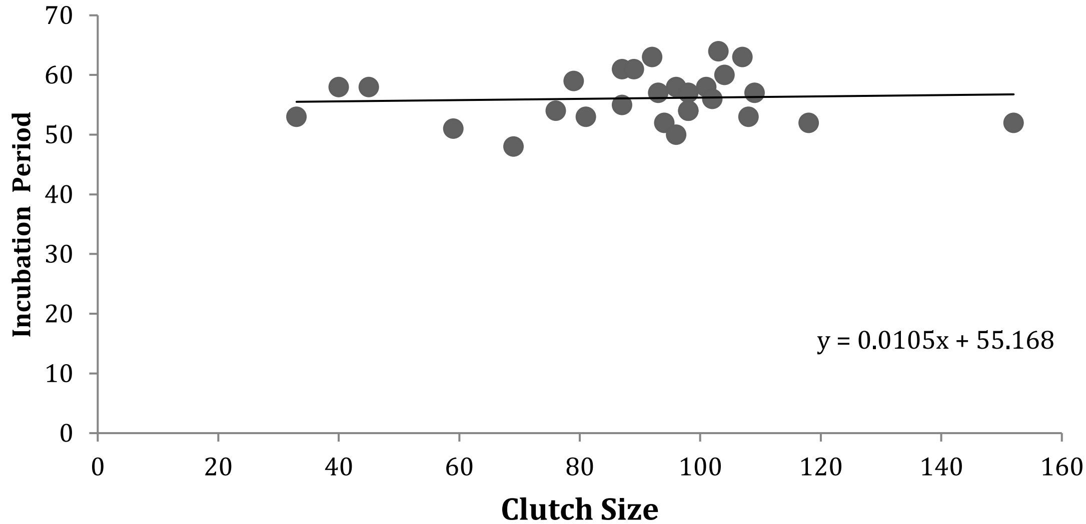

Figure 4. Correlation analysis of the influence of clutch size on the duration of incubation for 28 nests. R = 0.0613.

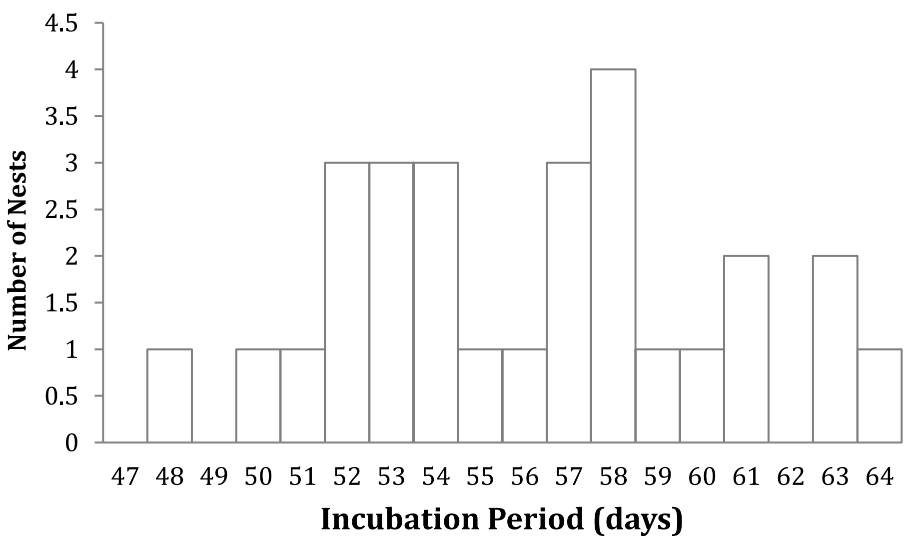

Figure 5. Distribution of incubation period for 28 nests from hatched data, ranging from 47 to 64 days.

Group A, B, and C

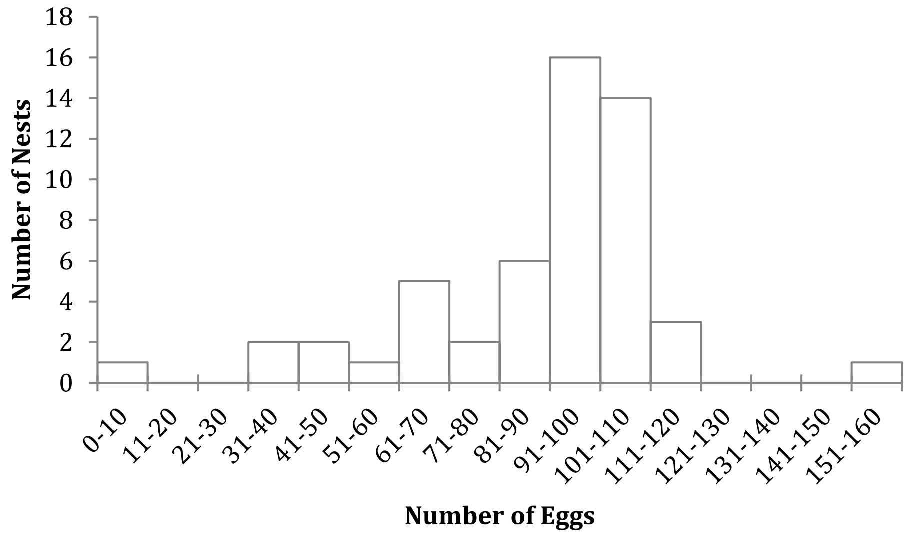

Figure 6. Frequency distribution of clutch size for 53 nests spanning Playa Junquillal, Honda and Estero beaches.

Discussion

Since its founding in 2010, the Playa Junquillal field station, Verdiazul, Costa Rica has channeled its energies and resources into constant monitoring and protection of endangered sea turtle populations and public education about the turtle species that visit the western Costa Rican coast [7]. The Leatherback, Green, and Olive Ridley turtles all travel to Playa Junquillal, Honda and Estero beaches to nest and lay hundreds of thousands of eggs over a single nesting season [2]. The course of this study included data from the Olive Ridley sea turtles nesting season and allowed for the evaluation of multiple factors that could influence the rate of survival and hatchling success.

The 13 nests from *Group A* allowed for the evaluation of the influence the phase of the moon and the tide have over optimal oviposition. 62% of the recovered nests were found during the full moon phase while the new, first quarter, and last quarter phases comprised 23% of nests discovered during patrols and no moon or low visibility represented 15% of nests recovered.

Interestingly, 84% of nests were laid during high tide. This result demonstrates a possible strategy of the turtle to conserve energy as well as to ensure appropriate positioning of nests. During high tide the turtles are able to utilize swimming as an efficient mechanism to land closer to the vegetation on shore. Thus, they only have to crawl a short distance through the sand to reach the vegetation and begin to make their nest. It also is reasonable to consider that the turtles use high tide to gather information about where to position their nest so it is not in a region where the tide, when transitioning from low to high will cause the nest to be flooded. The ratio of water, oxygen, carbon dioxide and other compounds in the nest is vital for egg development. If the nest is saturated with water the eggs will not develop and the embryos will not survive.

A sample size of 28 nests and 2,514 eggs constituted *Group B*. From this data, average clutch size was 89.8 eggs and incubation for the majority of nests ranged between 52-54 days or 57-58 days. Three of the studies examining the association between incubation period and number of live hatchlings, incubation period and percent of live hatchlings, and clutch size and incubation period, resulted in the conclusion that these factors had no correlation to hatchling success. However, when comparing the clutch size to the percent of live hatchlings released, a weak positive correlation with a correlation coefficient, R, of 0.156 was observed. While it is not clear whether the weak correlation is significant, further study into the correlation between clutch size and hatchling success is warranted. This comparison could provide important insights into the turtle population. Assuming that clutch size is indicative of the reproductive fitness of the Olive Ridley adult female turtle, a high number of eggs in the nest will represent a healthy female. The health of the adult female could be directly associated with the success of embryonic development and the percent of eggs that hatch. A healthy adult female will have a higher amount of energy to contribute to the production of eggs as well as sufficient amounts of nutrients to deposit in the yolk of the fertilized egg. The rich supply of nutrients will help to ensure full development of the embryos and thus result in higher percentages of hatchlings after incubation. Further support for studies to compare clutch size to the percent of live hatchlings would come if there were an environmental factor that created a level of toxicity and exposure to the toxin resulted in reduced health of the adult female. Toxin exposure could diminish the female's reproductive output, lead to nutrient deficient eggs, or interfere with embryonic development decreasing the percent of live hatchlings per nest. By examining the association between clutch size and percent of live hatchlings the developmental success of nests could be observed and the health of adult female turtles could be extrapolated.

Of the 2,512 eggs 2,042 hatched and 97% of the 2,042 were successfully released to the ocean. This value is higher than the *in situ* value of 33% emergence success represented in the literature [2]. The significantly higher hatchling success most likely stems from the protection provided against predation during incubation. The plastic net structures that were placed below and above the nests prevented any unwanted pest from disturbing or eating the eggs. From the unhatched eggs 12.8% were not fertilized and roughly twenty percent had reached stages three and four in development, described in the legend of Table 1. It is important to note that nests acting as outliers in the data could have been subjected to a number of natural factors including infection (bacterial or fungal) or exposure to an incubation temperature incompatible with development (thermal tolerance range (TTR) 25-35°C) [4]. 3.39% of the unhatched eggs displayed characteristics of infection including putrid odor and black growth distributed throughout the yolk.

A longitudinal study to build upon the data from this study could yield statistically significant support for factors influencing hatchling success. Identification of possible microbe growth in unhatched eggs would provide a more thorough analysis into possible reasons why certain nests had lower success hatching rates. Also monitoring and regulation of sand temperature during the incubation period of nests would contribute to the prevention of female-only offspring production as a result of temperature dependent sex determination. Though brief in duration the study marks an important contribution to conservation efforts for the endangered turtle species and preservation of the positive impacts they have on the oceanic and terrestrial environments.

Limitations

Limitations to the study included a shorter period of data collection. During this time only eight clutches hatched. However, I was able to obtain data from pervious clutches that had already been examined and released. The inability to identify possible microbe growth in unhatched eggs was also a limitation to the study; as it would have helped to further explain hatching success rates. A final limitation included the parameters set by the organization; restricting the study to the data that was made available during time spent at the Playa Junquillal field station.

Reference Images

Reference Image 1
A portion of the turtle hatchery with the 2’ by 2’ divisional grid and the plastic netting structures used to protect the relocated nests from predators.

Reference Image 2
Unhatched eggs collected from a newly released clutch. The unhatched eggs will be opened and examined.

Citations

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Appendix A

Group A

Moon Phase	Tide	Location	Clutch Size
Full	3/4 to high	Playa Honda	61
No moon	3/4 to low	Playa Estero	93
New	1/4 to low	Playa Junquillal	108
Last Quarter	1/4 to high	Playa Estero	99
First Quarter	1/4 to high	Playa Junquillal	98
No moon	1/4 to high	Playa Estero	0
Full	1/4 to high	Playa Junquillal	107
Full	1/2 to high	Playa Estero	103
Full	3/4 to high	Playa Junquillal	8
Full	3/4 to high	Playa Estero	91
Full	High Tide	Playa Junquillal	109
Full	1/4 to high	Playa Junquillal	87
Full	1/2 to high	Playa Junquillal	97

Appendix B

Group B

Clutch Size	Incubation Period	No Embryo	Percent Hatchlings
108	53	6	91.67%
59	51	2	93.22%
96	50	4	90.63%
118	52	26	63.56%
94	52	9	28.72%
103	64	11	80.50%
107	63	5	92.52%
104	60	37	62.50%
98	54	4	93.07%
45	58	10	77.77%
96	58	5	94.79%
40	58	34	2.5%
76	54	0	100%
33	53	5	84.85%
89	61	0	98.87%
101	58	0	97.03%
87	55	12	86.20%
152	52	23	84.87%
79	59	8	88.61%
98	57	63	18.36%
69	48	9	85.51%
109	57	3	96.33%
93	57	2	94.62%
87	61	28	62.91%
92	63	1	96.70%
81	53	2	93.83%
102	56	6	92.15%
98	54	7	89.47%

Appendix C

Group C

Beach Location	Number of Eggs
Junquillal	94
-	101
Estero	114
Junquillal	111
Junquillal	61
Estero	104
Estero	70
Estero	67
-	110
Estero	47
-	83
Junquillal	99