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Degree Final Project

Analysis of the effect of Sun / Shade treatment on the success of hatching, the emergency success, and the morphology of the specie Lepidochelys olivacea marine turtles, in a hatchery on the Piro beach

(Peninsula of Osa, Costa Rica).

Claudia Hurtado Pampín

Degree in Biology

Tutor: Dr. Lluís Benejam

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DEGREE FINAL PROJECT SUMMARY

Title: *Analysis of the effect of Sun / Shade treatment on the success of hatching, the emergency success, and the morphology of the specie Lepidochelys olivacea marine turtles, in a hatchery on the Piro beach (Peninsula of Osa, Costa Rica).*
Key words: *Lepidochelys olivacea, hatchery, emergence success, hatchery success, incubation temperature, sex difference.*
Author: Claudia Hurtado Pampín
Tutor/s: Lluís Benejam
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This study presents an incubation success' analysis (success of hatching and emergency success) of the Olive ridley turtle (*Lepidochelys olivacea*) on the beach of Piro (Osa Peninsula, Costa Rica), according to the different treatments that each nest received (sun and shade) in a hatchery built in the nesting season of 2017 (from June to December).

The main objective of the study is to evaluate if these different treatments had a significant effect on the biometric variable, the success of the nest (emergency and hatching), the hatching time and the different developmental stages of the embryos. For this purpose, it was determined if the use of a shady part in the hatchery was beneficial for the survival of the species. Considering that environmental and anthropological threats of the area affect the species, the changes planned in the environment a few years from now should also be taken into account.

To carry out these objectives, each nest in the hatchery was carefully observed and the incubation temperature was strictly controlled. The total of the turtles released in each nest was counted and the biometric measurements of a group of hatchlings were taken. Thanks to an exhumation that was carried out in all nests of the hatchery, it was possible to calculate the hatching and emergence success of each nest. Subsequently, the results were analyzed statistically with the program *R commander*.

The results obtained show that there were significant differences between sun and shade treatment for different variables like length and width of the hatchling, success of emergence and hatching, duration of the nest to be born and in two stages of the embryonic development (the 1th and 3th stages). Moreover, the incubation temperature influences nest survival. The analysis shows that the lower shade temperatures negatively affect the survival of the nest. In any case, considering the parameters of the beach, the morphology of the turtles and the effect of climate change in the area, the use of a shaded area in a hatchery would bring benefits for the survival of the species in a long term.

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1. Introduction

1.1. Biology and ecology

Sea turtles are all derived from the same ancestor of the *Cryptodira* suborder and are about 150 million years old. They have shown to have a life cycle and a high, successful and effective reproduction mechanism (Didiher Chacón, Juan Sanchez, 2009) . Until a couple of centuries ago, the worldwide population of sea turtles was abundant. Some of the populations were made up of millions of individuals and in the Eastern Pacific, they were considered among one of the world's largest. However, these populations have drastically decreased because of human activity during the decades of the 60s and 70s (Dinarzada, Alvarez, Dinarzada, & Alvarez, 2017).

The Olive ridley turtle *Lepidochelys olivacea* is found in tropical and subtropical regions throughout the world's oceans and is the most abundant marine turtle (Eguchi, Gerrodette, Pitman, Seminoff, & Dutton, 2007) (**Figure 1**). They reach sexual maturity between the age of 10 and 18 years. They are reproductively active for at least 21 years (Dinarzada et al., 2017). The spawning of this species takes place on land, which is an ancestral characteristic, and they do not give parental care of the hatchlings (**Figure 2**) (Didiher Chacón, Juan Sanchez, 2009).

Figure 1. Distribution of the olive ridley turtle, Lepidochelys olivacea. Source: *(Sandoval, 2008)*

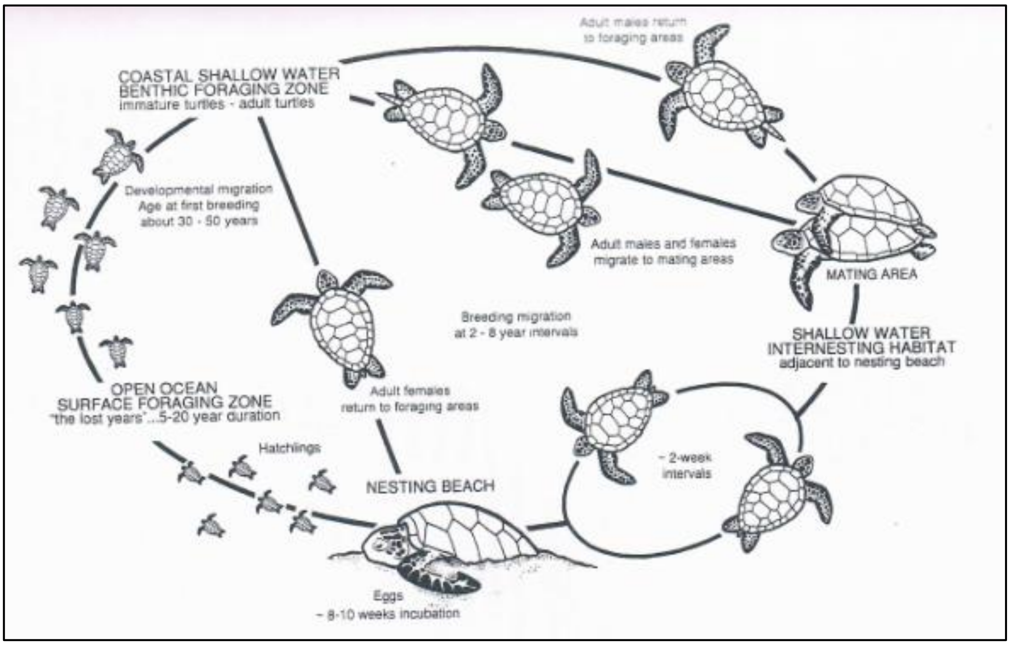


Figure 2. Generalized life cycle of sea turtles: individual species vary in the duration of phases. Source: *Lutz & Musick, 1997*.

The nesting peak of *L. olivacea* occurs between the months of July to December. They generally lay eggs in solitude more than twice every year with an average of 100 eggs per laying. The egg laying is along the Pacific coast, from Mexico to Ecuador. Thus, they also make the arrivals, which consists of a synchronized nesting of up to thousands of females. It takes place on a determined beach in a period of approximately 2 to 8 days (Playa Ostional in Costa Rica) (Endara, 2017). Despite the great abundance of arrival nesters, their numbers are comparable to those of solitary nesters, due to the expansive nesting range of the (Eguchi et al., 2007).

The hatchlings of this species are dorsal and ventrally dark grey to black. While the adults dorsally go from olive-grey to a yellowish olive and ventrally from beige to a light greenish grey, with dark spots on the ends of the fins. As to the dimensions of the eggs, they present an average diameter of 39,7mm and a weight of 34,2gr. The nests can contain from 54 to 189 eggs. Hatchlings reach an average carapace length of 43,5mm and a weight of 16,2g (**Figure 4**). In adults the carapace length is 51 to 78 cm and their weight, 33 to 52 kg (Sandoval, 2008) (**Figure 3**). In general, the size of the neonates increases with the size of the egg, which is generally correlated with the size of the yolk (Finkler & Claussen, 1997). The biotic characteristics of sea turtles, as well as the abiotic conditions and the environment of the eggs affect the development of the embryos (Sandoval, 2008).

Figure 3. Olive ridley turtle (Lepidochelys olivacea) laying the eggs on Piro beach. Source: Claudia Hurtado Pampín.

Figure 4. Hatchling of Lepidochelys olivacea in Piro beach. Source: Manuel Sánchez Mendoza.

This species generally selects beaches with high levels of humidity, most of them are near river mouths or estuaries (Casas Andreu, 1978; Márquez-Millán, 1996). These characteristics agree with the present study area. The purpose of humidity is twofold. Firstly, it acts as a cooling strategy against the elevated temperatures that can occur in the environment and secondly it increases the viability of egg development. The incubation is affected by varying conditions of humidity and it constitutes one of the main causes of mortality in marine turtles. However, several success models of emergence and hatching of the species *L. olivacea* eliminated the variable of precipitation, although the period of nesting and incubation of the eggs occurs within the rainy season. However, this variable does not seem to influence the incubation of this species (Dinarzada et al., 2017).

The species normally visit bays and estuaries, since these ecosystems are their main feeding areas due to the abundance of crustaceans and invertebrates. *L. olivacea* has a beak-shaped jaw that provides a diet based mainly on benthic organisms (Endara, 2017). In a study carried out on the coast of Oaxaca, the stomaches of this specie were examined and it was found that they fed on in order of importance: salps, fish, mollusks, crustaceans, algae, bryozoans, sipunculids, sea squirts and fish eggs (Sandoval, 2008).

Unlike most species of sea turtles that leave their specific feeding areas to go to their nesting areas, *L.olivacea* exploits several feeding areas before heading to their breeding grounds. Female sea turtles have a high fidelity to their region of birth and tend to nest on the same beaches where they were born or very close, which means they are phylopatric species (Dinarzada et al., 2017). They remember their natal beach by imprint or fixation, which means that when the neonates head towards the sea they register parameters that are printed in their memory to recognize the same beach (Didiher Chacón, Juan Sanchez, 2009).

All species of sea turtles have migratory behaviours during the different stages of development, fulfilling an extremely important role within the ecosystems in which they interact. Jackson (1997) mentions the importance of mega fauna in ecosystems, for the large amount of food they consume that generates physical and biological alterations intervening directly with other species. Not only do they play an important role in controlling the populations of other species, but they also transfer energy from one ecosystem to another. By nesting outside the water, the females transfer energy from marine-coastal zones to the beaches where they lay their eggs. The eggs, in turn, contribute towards the gain of organic matter in nutrient-poor soils (Meylan et al., 1985, Meylan, 1988). Therefore, the significant reduction of sea turtles creates an imbalance in the marine-coastal ecosystem, affecting all levels of the trophic chain (Endara, 2017). They are animals that have an important relationship with coastal and oceanic habitats (for example, they contribute to the health and maintenance of coral reefs, seagrass meadows, estuaries and sandy beaches) (GETM-IUCN, 2000) (**Figure 5**)

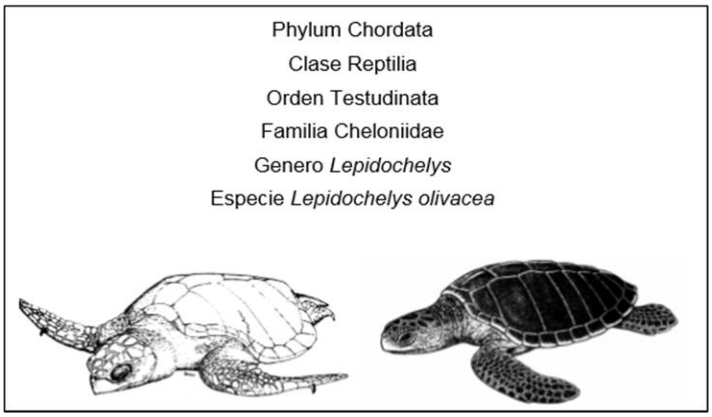


Figure 5. Taxonomical classification of the Olive ridley turtle Lepidochelys olivacea. Source: (Sandoval, 2008)

Like other marine turtles, Olive ridleys have been affected by a variety of human activities such as the direct harvest of eggs and turtles, nesting habitat destruction, and fishery bycatch. Because of these impacts, nesting abundances on many of the primary nesting beaches have declined to the extent that several Olive ridley arribadas appear to be an endangered phenomenon (Dinarzada et al., 2017). Due to these ongoing threats and ensuing population declines, the Olive ridley remains listed as 'Endangered' in the IUCN World Conservation Union Red List (IUCN 2006) and included in Appendix I of the Convention on International Trade in Endangered Species (CITES), which refers to the prohibition of international market. Despite a worldwide increase in research and conservation of Olive ridleys, little data is available on their density and abundance in most regions. Successful management of a population requires information about mortality, recruitment, and temporal changes in abundance or density. Longitudinal estimates of abundance or density are necessary for management and monitoring of marine turtle populations. For marine turtles, the annual number of females at nesting beaches has been used as an index of abundance because the proportion of the total females nesting in any season may vary substantially from year to year. An alternative and complementary method to nesting beach surveys is sampling at sea. However, only a few studies have used distance sampling methods to estimate marine turtle abundance or density at sea (Eguchi et al., 2007). The overexploitation of this species has decreased the number of turtles that arrive to nest from hundreds of thousands to a mere few thousand (Endara, 2017).

Sea turtles are decreasing drastically or in a process of decline in all areas of their global distribution. For this reason, there are more comprehensive programs aimed at preventing the extinction of species and promoting the recovery and maintenance of healthy populations of marine turtles. Sea turtles and humans have been linked since the period when humans settled on the coasts and began their tours of the oceans. For countless generations, coastal communities have depended on sea turtles and their eggs to obtain proteins and other products. In many regions, this practice continues. Most turtle populations have decreased due to unsustainable harvesting practices for the use of their meat, shell, oil, skin, and eggs. Thousands of turtles die every year when they are caught accidentally in active or abandoned fishing gear. In addition, many areas of nesting and feeding have been disabled or have a clear deterioration, due to petrol spills, accumulation of chemical waste, non-degradable plastics, and other anthropogenic waste. Moreover, the high impact coastal development and the increase of tourism also negatively affect the nesting beaches (GETM-IUCN, 2000). The relative abundance of Olive ridley turtles in relation to other marine turtles has also been severely reduced over time (Robles & Vega, 2007).

The population of the turtles is affected by a relatively low probability of embryo survival, as a consequence of the predation of eggs by humans, other animals and female turtles that destroy the nests. In addition, abiotic factors such as extreme temperatures and humidity, as well as microbial infection cause embryo destruction along with a low probability of adult survival (Sandoval, 2008).

Having knowledge about the natural sex ratio in nesting population of sea turtles is an important component of any management plan and it provides a point of comparison when assessing the effects of conservation techniques (Sandoval, 2008). Hatch and emergence rates should be estimated at priority beaches with high levels of nesting. In the cases where hatching or emergence is low, the most likely causes of threat should be determined as so to focus conservation efforts and to improve reproductive success (Dinarzada et al., 2017).

A project always needs an investment of human and financial resources and a community organization. For the functioning of these projects and their environmental laws, there must be a good coordination between governmental, non-governmental and communal institutions. In Costa Rica there is the SINAC, the National System of Conservation Areas, which is responsible for the regulation and the monitoring of conservation carried out in different areas (Didiher Chacón, Juan Sanchez, 2009). The main objective of protection programs should be to produce healthy hatchlings that have a greater chance of surviving, growing and increasing the species' population (Sarti et al., 2006).

In addition, global climate change is a major issue in ecosystem and wild life management throughout the world. It has already produced significant and measurable impacts in almost all ecosystems, taxa and ecological processes and its impacts are expected to increase rapidly. To respond effectively to the predicted climatic change it is important to understand how these changes will affect biodiversity (Fuentes & Porter, 2013). Projections of changes in global temperature, sea level rise, rainfall and increased intensity of hurricanes and storms in tropical areas due to global warming, will impact the life cycle and reproductive development of sea turtles (Dinarzada et al., 2017).

The history, physiology and behaviour traits of sea turtles are extremely influenced by environmental temperature. Thus, their distribution, reproduction and foraging ecology will likely be affected by a projected temperature increase. The most detectable impacts of climate change on sea turtles will likely be on their terrestrial reproductive phase since projected increases in temperature could alter their nesting phenology, the sex of the hatchlings and the success and duration of incubation. The successful incubation of sea turtle eggs occurs within a narrow thermal range of 25 °C to 33 °C. The prolonged exposure of eggs to their upper thermal threshold (~33 °C) increases the probability of morphological abnormalities and reduces hatching success and survival. Additionally, sea turtles exhibit temperature-dependent sex determination, therefore the incubation temperature determines the sex of hatchlings (TSD) and warmer temperatures produce higher proportions of females. The transitional range of temperature (TRT) is the range of temperature in the nest in which sex ratio shifts from all males to all females. And the pivotal temperature (PT) is where a 50:50 male to female sex ratio is produced. A high degree of feminization in turtle populations could alter reproductive factors (e.g. intra- and intersexual competition, sperm competition, and multiple paternity) and lead to rates of fertilization and loss of genetic variation, thereby jeopardizing the ability of turtle populations to adapt to climate change (Jourdan & Fuentes, 2015). The sensitive period for sex determination appears to occur around the middle third of incubation (Lutz & Musick, 1997) (**Figure 6**).

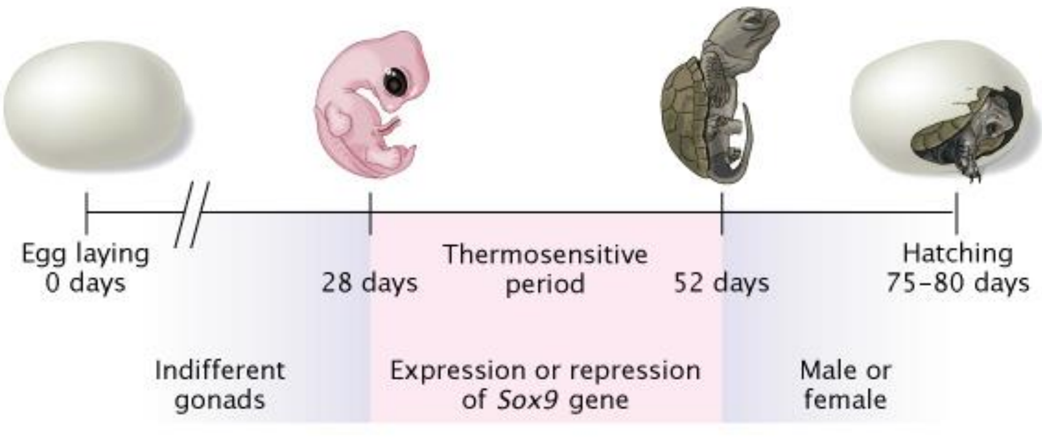


Figure 6. Hatchling embryonic development. Source: (Pieau, 2004)

Concerns about the potential impact of increased environmental temperature on sea turtles have prompted the identification of management strategies to mitigate potential threats on their terrestrial reproductive phase. Strategies include manipulation of incubation, temperatures using shade, water sprinklers, native vegetation, and the addition of sediment with different colour and grain. However, little is known about how effective these strategies are at reducing sand temperature and therefore mitigating potential impacts of climate change on sea turtle's reproductive output (Jourdan & Fuentes, 2015).

Additionally, it is necessary to consider the fact that they are long-lived organisms with a late age of first reproduction, as well as a prolonged reproductive potential. This last characteristic implies that the production of hatchlings will not impact on the recruitment of the population of nesting females, but until a period of decades after the hatchlings of their hatching beach (Robles & Vega, 2007).

1.2. Hatchery as a management tool

As a result of the threats for these animals that we have seen in the previous section, usually the best solution is to relocate the eggs to a safer and controlled place such as hatcheries. Hatcheries are a delimited area of the beach where nests are relocated to give more survival to the species.

In Costa Rica the main problem is the illegal recollection of eggs, as well as their predation and the erosion of the beaches. The best solution is to usually move the nests to safer places and erase the footprints of the nests. It is always recommended to use hatcheries, which always increase the percentage of success. It is known that 1 of every 1000 turtles that are released actually survive, that is why these measures must be taken. It is important to be careful not to monosex the population, which could cause damage to the ability to perpetuate (Didiher Chacón, Juan Sanchez, 2009).

There is controversy about the utility of the nest transferring and the elimination of in situ protection hatcheries, because the management of the nests can cause a lower success of hatching and an alteration of the hatchlings' sex (Sandoval, 2008). Nevertheless, hatcheries are a successful tool against the looting of nests and animal depredation, and they also offer benefits as instruments of environmental education (Dinarzada et al., 2017).The new location of the eggs must have adequate conditions of humidity, temperature and gas exchange to support the development of the embryos and keep them safe from predators and poachers. Temperature monitoring is extremely important to understand the incubation environment, even if the relocation of the eggs is an option for conservation (Robles & Vega, 2007).

In Costa Rica there is an intensive effort to conserve eggs through hatcheries. However, some operate as a mean of conservation effort and not as a tool. For this reason, many things are not done correctly in hatcheries, such as (Didiher Chacón, Juan Sanchez, 2009):

- Egg relocation models in hatcheries are not based on percentages obtained from an analysis of the population condition and its capacity to withstand any type of impact. They are more models that correspond to seemingly unpredictable decisions.
- There are no state parameters to regulate the correct operation procedures of hatcheries. Standard methodologies are not used, nor knowledge renewal.
- The situation reveals that there are no regulations to rule the handling of eggs and of hatchlings and the integral operation of the hatchery.
- In appearance the hatcheries are reflecting actions that favor the comfort of those who operate them and do not correspond to duplicate the natural conditions that the turtles require.
- Hatchery workers are leaving aside vital information related to the sex of the newborns and their relationship with the work of the hatchery.

With the use of hatcheries, advantages for conservation are obtained. It is possible to document the success of hatching and to know the influence of factors such as humidity, temperature and handling. The production of neonates and the monitoring of the incubation period can help to generate technical knowledge and public awareness. Increases nest performance and allows to apply performance indexes for the project. It is a space to coordinate conservation actions as sites for the control of access to the beach and as a demonstration area specially to demonstrate the financial investment in conservation. It serves as a "demonstration area" and involves various kinds of public (education, public information) (Didiher Chacón, Juan Sanchez, 2009).

On the contrary, there are also a series of disadvantages. Since it is an expensive method for the material needed and the personnel of monitoring, a procedure of high risk by the waves, the tides and storms. It requires trained personnel. Percentages of success are usually lower than natural nests. The absence of proven methodology or monitoring can increase the mortality of the species. All the nests undergo a similar environmental treatment, impacting on the sex ratio in the neonates. The nests are prone to the infection of bacteria, fungi, insects, etc. which requires intense sanitary processes. There is an increase in the compaction of the sand and therefore a decrease in oxygen, causing deformities and difficulty of intuitive exit of the nest by neonates. And finally, they induce absolute protectionism (Didiher Chacón, Juan Sanchez, 2009).

Taking everything into account, the contribution of this study is to highlight the influence of temperature, which is one of the environmental variables that affects the most in the success of hatching and emergence in hatchery conditions. Working with turtles of the species *L.olivacea* and in a separate hatchery in two zones, one placed in the sun and one in the shade.

2. Objectives

The main objective of the study is to evaluate if the Sun / Shade treatment in a hatchery has a significant effect between the different studied variables of breeding success of the species *L.olivaceia* (Olive Ridley).

This study analyzed the effect of the treatment in the biometric variable (length, width and weight), the success of the nest (release, emergency and hatching), the hatching time and the different stages of embryo development. With these results we could extrapolate what could happen in the study area with the increase of temperature due to climate change in the following years in order to reach a conclusion on the methodology to be followed for guaranteeing the maximum survival of the species in a long term run.

3. Methodology

3.1. Study Area

The present study was conducted at Piro Beach in Osa Peninsula, which is located jutting into the sea at the southern end of Costa Rica's Pacific coast (**Figure 7**). Piro Beach is known to be one of the most important nesting grounds for Olive ridley turtles in the Pacific Ocean. This population has an important ecological role, social importance to indigenous people (Campbell, 2003) and value to the tourism industry (Kobayash et al., 2017). That is the reason why working in this area is crucial for the conservation of these threatened and vulnerable species. In this part of Costa Rica, there are lots of threats, mainly for the illegal marketing of eggs. Furthermore, they should deal with the erosion and predation.

Figure 7. On the top a map from Costa Rica and Osa peninsula. to low, map of the beach where we carry out our study. Source: “About the Osa Peninsula,”

3.2. Hatchery

A previous study about the background of the beach was necessary to choose the best place to build the hatchery at the beginning of the peak nesting season in June 2017. In order for the hatchery to be prepared for that season, it began to be built at the beginning of May 2017. While it was being built, the last season one was used. It was located in another place near the beach. The hatcheries need to be in separate places after each season with the possibility of returning to the same place after a period of two years. The reason why it is not possible to reuse the hatchery is because when neonates open the egg shell, a significant amount of amniotic fluid spills into the sand. Therefore, the contamination of the sand is a means of attraction for invertebrates such as saprophagous flies, ants and cockroaches as well as an excellent growth environment for the development of microorganisms such as fungi and bacteria (Didiher Chacón, Juan Sanchez, 2009).

The following factors were taken into account: the behaviour of the tides, the dynamics of the rivers mouths and natural drainage, the impact of erosion, the distribution of past nesting and the areas with less organic matter load. Thus, the chosen place was at kilometre 1.8, close to the middle of the patrol's route. The hatchery was provided with air circulation and a porous canvas that allowed the raindrops to go in. In order to protect it, a 1.2 meters bamboo-stick fence was made (**Figure 10**).

The hatchery was run by a group of biologists and the turtle coordinators of the Osa Conservation Project. Osa Conservation is a local nonprofit organization dedicated to protecting the globally significant biodiversity of the Osa Peninsula, Costa Rica. They were in charge of finding the optimal place, the necessary materials and the financial and physical help of volunteers and associations that could contribute so that the project could be carried out year after year ("La conservación del tesoro natural de Costa Rica - Conservación Osa,").

The size of the hatchery depends on the previous season number of nests planted. The location of this hatchery must be far from the shore, with good access for workers and in the middle of the beach in which the study was taking place, to ease the relocation of the eggs in the shortest possible time. In the hatchery, each nest must have an area that was not less than 1 nest/m2 and there should be an order (Didiher Chacón, Juan Sanchez, 2009). It was used the matrix method where the hatchery is divided into rows and columns built with ropes. Numbers are assigned to lines, letters to columns. Between nests a gap must be left empty, so neighboring nests temperature is not affected (**Figure 8**). Our hatchery was divided into two zones: sun zone and shade zone. The sun area was covered by a net of protection to avoid predators (e.g. birds, coatis, racoons, crabs and dogs) . It had a large mesh size that allowed the sun to enter naturally. Nevertheless, the shade zone was the other half of the hatchery and it was covered on the top. Apart from having a net, this area also had a porous opaque mesh size that did not allow the sunlight but the raindrops (**Figure 9**).

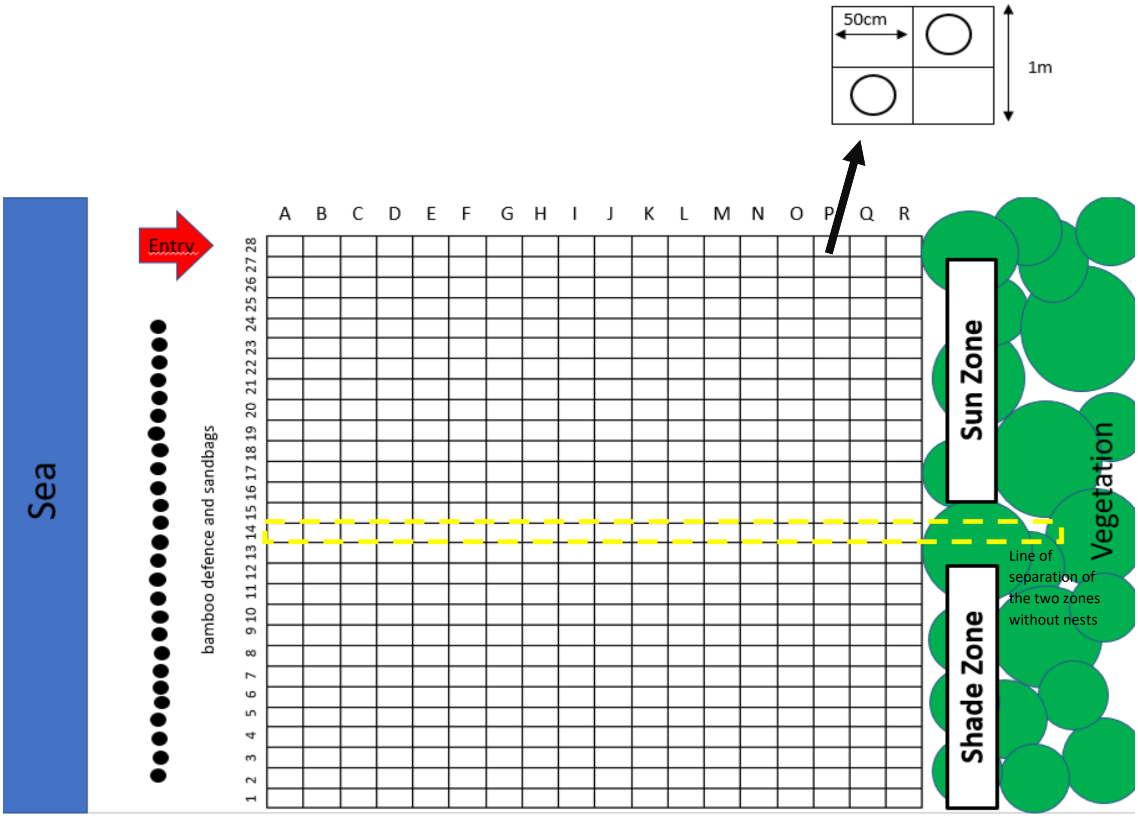


Figure 8. Hatchery diagram Source: Claudia Hurtado Pampín.

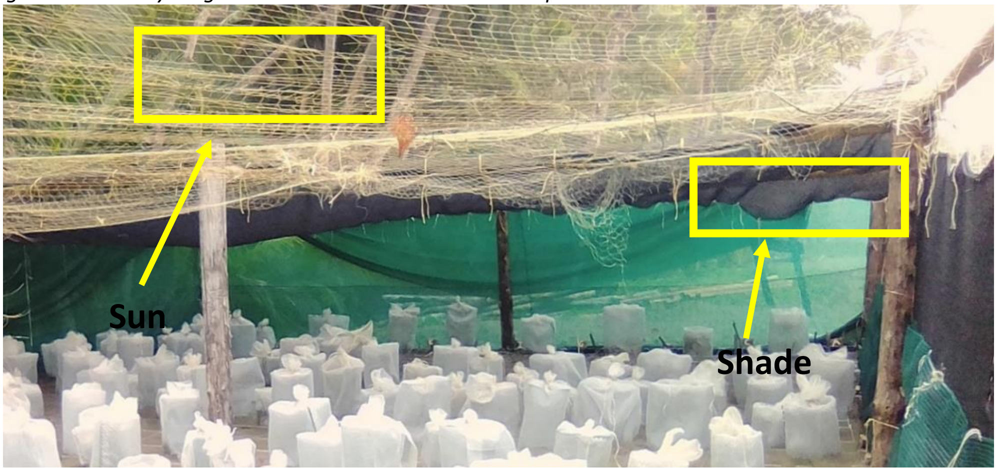


Figure 9. Shade and sun zone in the hatchery. Source: Claudia Hurtado Pampín.

Figure 10. Hatchery. Source: Claudia Hurtado Pampín.

3.3. Data collection

I spent the months of September, October, November, and the beginning of December 2017 working in this project of sea turtles and collecting data for the study. Field work was about working in a turtle hatchery where eggs of Olive ridley turtle nests were relocated to the beach during the patrolling work. Two and a half kilometres from this beach were patrolled every dawning and some nights. Hatchery nests consisted of eggs that were transferred to the hatchery immediately after nesting females had returned to the sea or as soon as the nests were found. These eggs were buried in artificially dug nests (Maulany et al., 2012).

During the patrol all the tracks from turtles that were found, were studied: the species were identified, if the turtle was still laying eggs or not, and if there was nest or was a false attempt. The maximum number of nests was relocated, giving priority to those that were in a bad area: near the shore of the beach where the tide arrives, near the river, places with a lot of erosion, near exits of trails where there was passage of people and places where there was a lot of predation. Once the trail was classified and decided that it would be a relocated nest, the nest had to be found and carefully excavated until the eggs were found. Then, the eggs were collected in a bucket in the same position where they were found, without turning them around, because otherwise we could alter or harm the unwinding of the embryo that would already be starting (Didiher Chacón, Juan Sanchez, 2009). The number of eggs were counted, 20 eggs from the first 80 nest were weighed and the width and depth of the nest were measured. The eggs of the same laying should be developed together, that is why they were planted together in the new nest of the hatchery, in which the measurements of the natural nest were used to build it to avoid a greater alteration of the process and to try to represent the maximum natural conditions. It was necessary to note the position of the nest, the number corresponding to the row and the letter corresponding to the column where it was. This process must be done carefully and the nest can never contain dry sand because it absorbs the mucus of the eggs and this can dehydrate them (Jourdan & Fuentes, 2015).

Once the eggs were buried and covered with sand, a basket was put on. It consisted of a cylindrical plastic net opened at both ends, of 60-70cm in diameter, height of 50-60 cm and a mauve light of 0.5x0.5cm (**Figure 11 and 12**). It also contains an outer lining to prevent the entry of saprophage flies or crabs, and buried 10 cm deep around the nest. In this way, we could collect the hatchlings at birth, which allowed an accurate calculation of the percentage of nest revival (Didiher Chacón, Juan Sanchez, 2009).

Figure 11. Baskets and hatchery inside. In the background the shadow zone and in front of the sun zone. Source: Claudia Hurtado Pampín.

Figure 12. Building the baskets for the hatchery. Source: Claudia Hurtado Pampín.

The hatchery was monitored every 6 or 8 hours at least 3 times a day if the weather allowed it (**Figure 13**). Each visit to the hatchery consisted in checking if any turtles had been born and to make sure nothing had altered the development of the eggs. In the case of found hatchlings, they were counted and the first 20 hatchlings from each nest were weighed. The width and length of their carapaces were measured and the morphology was examined (**Figure 14**). These variables would be related to the locomotor and swimming capacity that these animals had. Once finished with the collection of biometric data, the hatchlings were transported in a bucket and released on the beach, each time in a different point and always in groups (**Figure 15**). They should never be released in less than 6 meters from the sea and they should be left alone on their way to the water, to ensure they have enough time to establish the necessary parameters to return to their natal beach and to exercise locomotion and movement of their fins. Normally, neonates are born in the first hours of darkness, as a strategy by predators. That is why it is recommended to make the releases at night or in hours without much sun, when the temperature is lower and there are no birds or predators (Didiher Chacón, Juan Sanchez, 2009)

Figure 13. Hatchery check. Source: Claudia Hurtado Pampín.

Figure 14. Measuring and weighing hatchlings. Source: Claudia Hurtado Pampín.

Figure 15. Turtle releasing. Source: Claudia Hurtado Pampín.

Also on those visits, a control of the temperature was made in 4 hatchery nests, two in the sun and two in the shade, chosen randomly. The temperature was measured by a thermometer with an accuracy of 0.2 ° C (GETM-IUCN, 2000), which was used until the hatchlings were born and then it was changed to a new nest from the same area. The thermometer was always buried in the same nest at 45cm depth. The selected nests were B2, D2, I11 and P10 for the shade, and C28, K26, E18 and 018 for the sun. When handling turtles and eggs, gloves were used for safety.

The temperature of the nests was measured from the beginning of August to December in different moments of the day, in the hours in which the temperature changed the most (6:00 am, 14:00 pm and 22:00 pm). During the day more samples were collected than at night due to the difficulty of accessing the hatchery at some moments. On the other hand, the weather (heavy rains, floods, and high tides) made it difficult to collect data some days because it prevented the arrival to the hatchery.

After three days from the birth of the first hatchlings in the nest, an exhumation was carried out (F**igure 17**). It consisted on a process in which the nest is dug to check what remained inside and if there were any alive turtles that could not reach the surface. After having done the exhumation, a counting about the following was carried out: shells, alive and dead turtles found on the surface, inside the nest, hatched but not succeed in leaving the shell (**Figure 18**) and unhatched eggs. The latter were classified into 5 states according to the space that the embryo covered in the egg amniotic cavity: undeveloped (embryo that had not started its development), Stage I (from 0 to 25%), Stage II (from 26% to 50%), Stage III (from 56% to 75%) and Stage IV (from 76 to 100%) (**Figure 16 and 19**) (Didiher Chacón, Juan Sanchez, 2009).

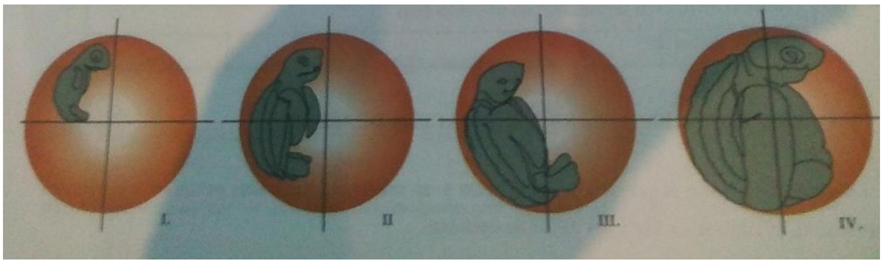


Figure 16. States of embryonic development of turtles. Source: (Didiher Chacón, Juan Sanchez, 2009)

Figure 17. Exhumation. Source: Claudia Hurtado Pampín.

Figure 18. The turtle hatched but not succeed in leaving the shell. Source: Manuel Sánchez Mendoza.

Figure 19. Example of an egg in state 3. Source: Claudia Hurtado Pampín.

Every day all this information was collected and recorded in the field notebook with another data like the date of the night the turtle had laid the eggs, the date in which it was calculated that the hatchlings were supposed to be born -for this species the approximate time was between 40 and 60 days (Kobayashi et al., 2017)-, and the date when the nest was finally born was also included.

3.4. Data analysis

Once the field work had finished, the analysis of the data was carried out, where first all the necessary variables for the study were calculated:

- Percentage of success: number of neonates in relation to the number of eggs
- Percentage of emergence: total of hatchlings that had emerged from the nest by themselves in relation to the total eggs in the nest.

- Hatchling Success: the hatching success is the number of hatchlings that were born in relation to the total number of planted eggs. This variable was obtained due to the exhumations.

The cause, which is the explanatory and independent variable in the study, was the area where the nest was located: sun or shade. The effect of this variable was used to test the effect on the dependent variables and it was studied together with the analysis of the data.

A first exploratory analysis was done with programs such as Excel and R commander to check that all the data was correct and to get an idea of where the results would go to know how to work with the data. It had to be checked if all the variables studied complied with the assumptions of normality of the parametric statistics. Therefore, we looked at the normality of the dependent variables with histograms, box diagrams and with the Shapiro-Wilks normality test. This test indicates if there is a normal distribution or not. The homogeneity of the variances was studied with cash diagrams by groups and with the Levene homoscedasticity test. Its objective was to check if the variance of the dependent variable was equal in our two groups.

We tried to make transformations of the variables to make the data and the errors adjust to the normal distribution. The transformations were made in order to improve the homogeneity of the variances, to reduce the influence of outliers and to improve the linearity. However, after the transformations, they still did not meet the requirements. For this reason, our initial data did not comply the assumptions and therefore it was analysed with a descriptive statistic: the Kruskal Wallis test. This non-parametric method which tests whether samples originate from the same distribution, it is used for comparing two or more independent samples of equal or different sample sizes.

Through the Kruskal-Wallis test, the mean ranks were examined to see if they were the same in all our groups ("Kruskal–Wallis test - Handbook of Biological Statistics," 2015). Always checking the comparison between the sun and shade average. The analysis was done with all the dependent variables, firstly with the biometric variables (Egg weight, Hatch Length, Hatch Weight, and Hatch Width), emergency success, number of releases, hatching success and hatching time. Secondly, it was done for the different stages of development of embryos. And finally, the same test was used to check if the difference of temperature was significative between our two groups: the sun and shade area (Maulany et al., 2012). And the box diagrams and histograms were used to visualize the results and the differences between both groups.

4. Results

During the 2017 peak nesting season (August-November), 146 nests were studied. The nests were divided in a hatchery with two different areas: sun zone and shade zone. The temperature was monitored in all nests.

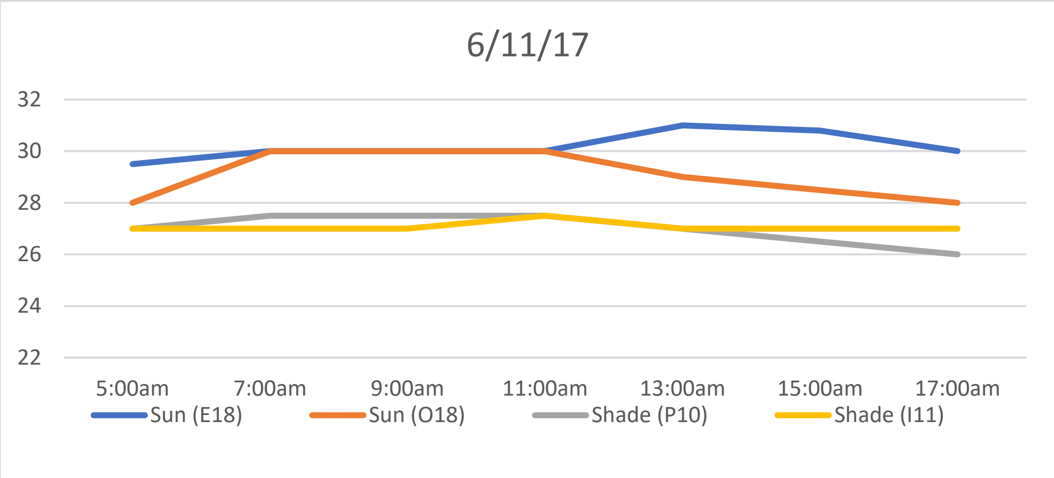
4.1. Temperature

The average temperature in the turtle nests was significantly different depending on the zone. Higher temperatures were found in the sun area (**Table 1**). There was always more than 1ºC of difference, in all the moments of the day. But the highest difference was at midday, at 2:00 PM when the temperature in the sun was 29,298ºC and in the shade 27,125ºC. This different was more than 2 degrees. The differences between the two zones of hatchery (sun zone or shade zone) was enough to consider that the conditions for the study were different depending the hatchery zone.

For a better understanding, two days of November, the temperature was measured every two hours to observe how it changed during the day, from 5am to 5pm. Lower temperatures were observed during the sunrise, increasing along the day and reaching the highest peaks of temperature at midday to go back down afterwards. All the nests followed the same temperature pattern (**Figure 20**).

VARIABLE	**Nº. samples**	**p-Value**	**Shade treatment average**	**Sun treatment average**
Temperature at 06:00 am	366	**<0,005**	26,995	28,939
Temperature at 02:00 pm	349	**<0,005**	27,125	29,298
Temperature at 10:00 pm	152	**<0,005**	27,468	29,118

Table 1. Analysis of the temperature in the different moments of the day, at the both zones. Source: Claudia Hurtado Pampín.



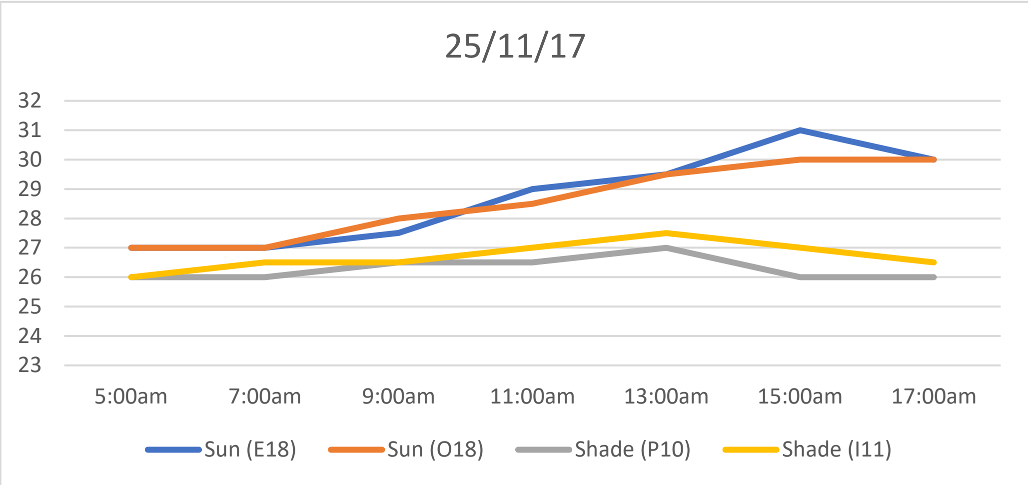


Figure 20. Graphic of two days where the temperature was measured every two hours. Source: Claudia Hurtado Pampín.

4.2. Biometric variables

In the first 80 nests collected, the weight of 20 of the eggs in each nest was measured. Therefore, 1574 samples were obtained. There was not any significant difference in terms of weight between sun and shade (Table 2).

Twenty hatchlings chosen randomly from their nests were weighed and the width and length of its shell were measured. Thus, more than 2500 samples of each variable were obtained.

In terms of width and length, a significant difference was observed depending on the treatment, especially in the length of the carapace. Regarding the length, the results were more varied (**Figure 21**). Thus, the turtles that had been placed in the sun were way longer than those in the shade. On the contrary, the width of the carapace was wider in the shade than in the sun. No significant difference was observed in relation to weight (**Table 2**).

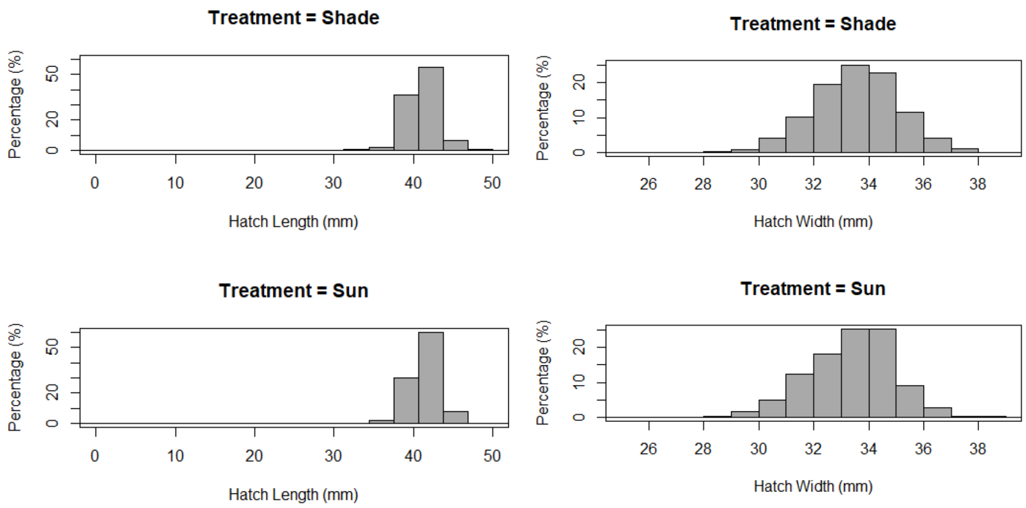


Figure 21. Histograms of the hatch length and width in the two groups: Sun-Shade. Source: Claudia Hurtado Pampín.

4.3. Success: Release, Emergency and Hatching

The variables of release, emergency and hatching success began to be taken when the first nest was born in September 17, 2017. They were calculated for all the nests until the study was finished at the beginning of December of that year. There were 145 studied nests and therefore, this was the number of samples that was obtained.

The release success of the nests did not have significant differences between the sun and the shade (Table 2). The number of hatchlings that was released in both zones was similar. However, for the other two variables significant differences were observed between the groups.

The emergency success had very significant differences. Then, it was seen that in the sun area, many more turtles emerged to the surface by themselves than in the shade. The same happened with the hatching success, it was higher in the sun (**Table 2**).

For these two variables, the box diagrams below show more varied results in the shade than in the sun. In the latter, the average is higher and the results are more concentrated. (**Figure 22**)

4.4. Hatching time

In addition, the hatching time of the nests had a very significant difference as well. For this species, when the eggs were laid, 40 to 60 days were calculated for them to be born.

It was observed that it took more time for them to be born when they were in the shade, about 12 more days. The eggs placed in the sun were born inside the range, in the shade it was exceeded by about 9-10 more days approximately. (**Table 2**)

For the two groups, the same number of different results was observed. The box diagram shows differences in the average and that the shade has a slightly more centred one. (**Figure 22**)

VARIABLE	**Nº. samples**	**p-Value**	**Shade treatment average**	**Sun treatment average**
Egg weight (abs)	1574	0,075	34,557	34,962
Hatch Length (abs)	2567	**<0,005**	41,010	41,202
Hatch Weight (abs)	2531	0,394	18,265	18,278
Hatch width (abs)	2567	**0,041**	34,008	33,863
Emergency Success (%)	145	**<0,005**	0,747	0,847
Released Success (%)	145	0,125	0,848	0,904
Hatchling Success (%)	145	**0,009**	0,821	0,896
Hatching Time (abs)	134	**<0,005**	69,531	57,543

Table 2. Analysis of the biometric variables, emergency success, number of releases, hatching success and hatching time. Source: Claudia Hurtado Pampín.

4.5. Different stages of development of embryos.

For the 136 unhatched eggs that we obtained in the study, an analysis was made. It was observed that there were no significant differences in the amount of them in the two zones.

Consequently, we analysed the different states of development of embryos. These analyses were carried out for each variable in an absolute value and in two percentages: One out of the total number of eggs and the other was calculated out of the total number of unhatched eggs to make sure if there were differences or not and to check the results in both ways.

Only significant differences were observed between the groups in State 1 and State 3. The latter had significant differences in the three cases analysed. However, the State 1, only had a relevant difference in the percentage of the total number of eggs, but it did not have any for the percentage on the unhatched eggs (**Table 3**). Probably, because in State 1 there were no significant differences between the groups in the number of unhatched eggs and possibly there were some in State 3. As a result, the percentage variables on the total eggs for these two States were studied. The average was always higher in the shade. In both States a similar diagram of boxes was observed, clearly showing a small amount of values in the sun in relation to a larger diagram found in the shade. (**Figure 22**).

VARIABLE	**Nº. samples**	**p-value**	**Shade treatment average**	**Sun treatment average**
Unhatched (abs)	136	0,186	7,101	3,566
Unhatched (%total eggs)	136	0,163	6,678	3,193
State 1 (abs)	136	0,074	0,710	0,237
State 1 (%total eggs)	136	**0,038**	0,675	0,171
State 1 (%Unhatched)	136	0,056	0,156	0,020
State 2 (abs)	136	0,263	0,406	0,210
State 2 (%total eggs)	136	0,263	0,382	0,206
State 2 (%Unhatched)	136	0,373	0,156	0,020
State 3 (abs)	136	**<0,005**	0,638	0,050
State 3 (%total eggs)	136	**<0,005**	0,600	0,085
State 3 (%Unhatched)	136	**<0,005**	0,079	0,004
State 4 (abs)	136	0,218	0,391	0,158
State 4 (%total eggs)	136	0,235	0,378	0,126
State 4 (%Unhatched)	136	0,219	0,037	0,009
Undeveloped (abs)	136	0,637	3,696	2,855
Undeveloped (%total eggs)	136	0,568	3,296	2,603
Undeveloped (%Unhatched)	136	0,395	0,606	0,277

Table 3. Kruskal Wallis test results of different embryo development stages. Source: Claudia Hurtado Pampín.

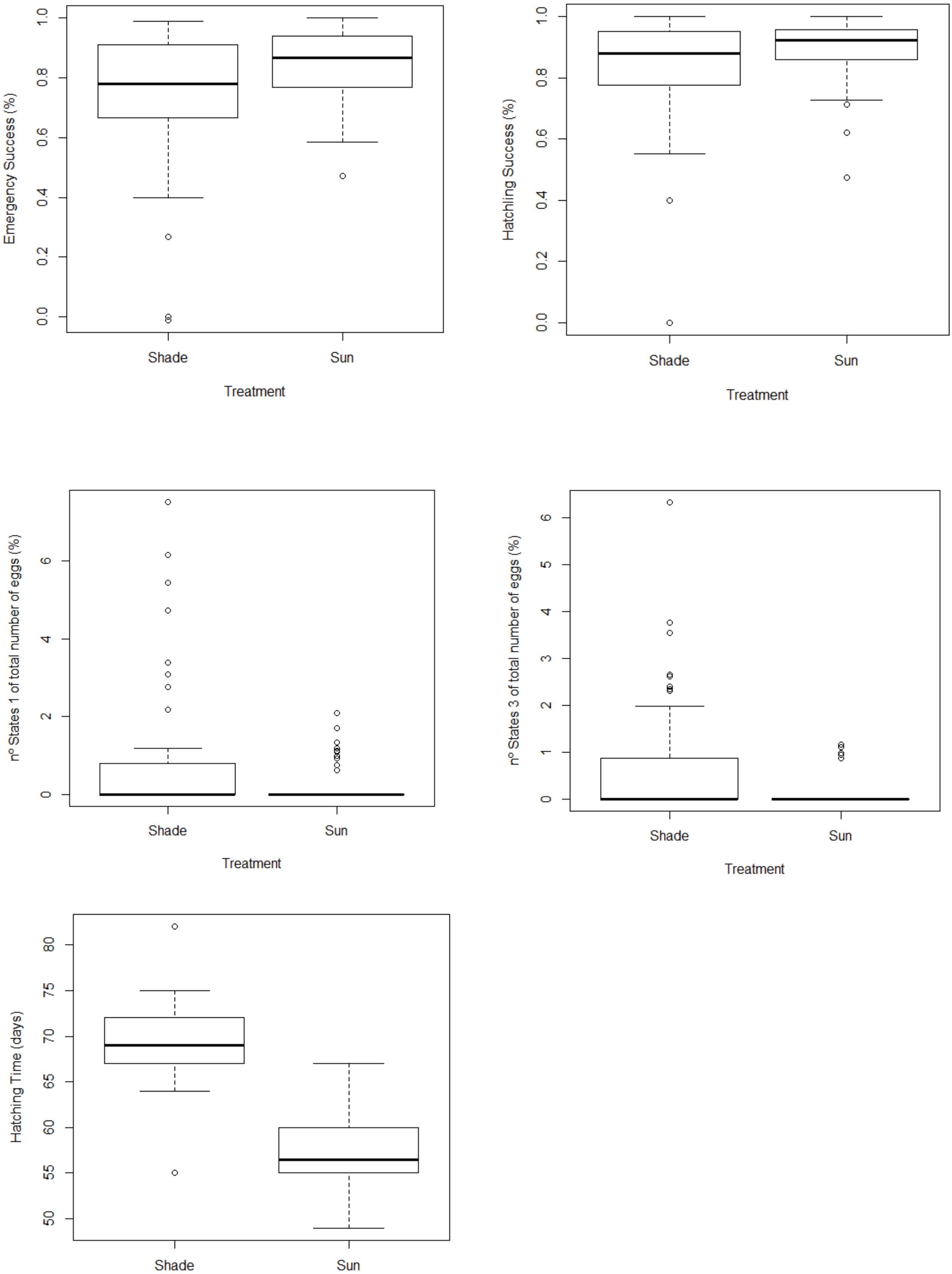


Figure 22. Box diagrams of all the variables with significant results: Emergency Success, Hatchling Success, nº State 1, nº State 3 and Hatching time. Source: Claudia Hurtado Pampín.

5. Discussions

In the present study, the effect of the treatment of sun and shade in a separate hatchery was studied. This separation is made to have temperature differences between the zones and thus not to monosex the species. To check the effectiveness of this methodology, it was examined whether the treatment in the sun or the shadow caused differences in the dependent variables discussed below. They could have a negative effect that would lead not to improve the conservation of the species, but without decreasing their survival. Differences were observed in the size of the hatchlings, in the success of emergence and hatching, with more success in the sun zone. On the contrary, the hatching time of the nest was highest in the shade zone. Finally, differences were also obtained in some states of development of the embryo (State 1 and 3). More unhatched eggs were found in State 1 and 3 in the shade. Higher differences in State 3 where embryonic development was more advanced and where the sex determination of the embryo took place. A similar amount of eggs was found in State 1 and 3 in the shade. In contrast, fewer eggs in the sun were observed in State 3 than in State 1.

5.1. Emergency and hatching success

The nests of sea turtles typically have a high hatching success (80% or more), but the external factors that affect them (predation, environmental changes or microbial infections) can affect the hatching (Dinarzada et al., 2017). That is why in many conservation programs the use of hatcheries is proposed as a tool for the survival of the species.

However, there is plenty of controversy about whether the hatcheries help or not in the survival of the species. The hatcheries are proposed as a tool for conservation and protection of the nests against all the threats they have. But at the same time, it is known that the handling, the movement and the transfer to other places with other conditions, can have negative effects on the embryonic development of eggs decreasing the survival of the species. For this reason, there are many studies that observe the success of nest survival in situ and in hatchery. With this observation they obtained information about whether the use of hatcheries is a good conservation strategy in the studied area. In addition, in the present work there has been studied whether the fact of having a part of the sun and another of shade also influences the success and survival of the species. Therefore, the evaluation of incubation success is necessary to know if a good performance is being done with these strategies or not. The evaluation of the success of the incubation is a two-step process consisting of determining hatching and emergence success (GETM-IUCN, 2000).

The emergency success refers to the number of hatchlings that reach the surface of the beach (the number of shells minus the number of live and dead hatchlings inside the nest) (GETM-IUCN, 2000). One of the main factors influencing the average of emergency success is the presence of advanced embryo-mortality that can be attributed to micro-environmental characteristics. The temperature of sand and humidity are two of those factors. A result of a 75-80% success, indicates that there is a good survival of the species (Dinarzada et al., 2017). In our results it was observed there was a higher emergency success than the interval stated above (84.7%) in the sun area as many of the turtles emerged by themselves to the surface without any problem or help. In contrast, in the shade area, there was a lower result than in the sun (74,7%), almost beating the borderline of the stablished interval of a good survival of the specie. As observed, the percentage in the shade shows a good success, but not as high as in the sun. A similar study conducted in Japan showed different results (Kobayashi et al., 2017). Since the effect of incubation temperature on embryos and hatchlings was not observed, the emergency success was similar in both areas. However, contrary to our study, the Japanese investigation did not use a hatchery to look at the difference between zones, but it studied them in situ. This could be the reason why they obtained different results. Perhaps the effect of shade in the hatchery was stronger and more effective than those nests found in situ. (Kobayashi et al., 2017)

Hatching success refers to the number of hatchlings that hatch or break their shell (same to the number of empty shells in the nest) (GETM-IUCN, 2000). A nest is considered to have a good survival when its hatching success is above 80% (Lutz & Musick, 1997). As the results of the present study show, it is seen that in both areas this percentage was exceeded (82.1% in the shade and 89.6% in the sun). Equivalent results were observed in the previous study carried out in Japan. The effect of the incubation temperature did not lead to hatching percentages below that established in either of the two zones. Nevertheless, the results were different to our study. In the one conducted in Japan, it was observed a lower percentage in the sun than in the shade, concluding that the elevated temperatures had a negative effect. This may be caused because in the study carried out by Kobayashi, the nests of the sun area were in situ whereas in our study, they were in a hatchery located in a high area, close to the vegetation. In the hatchery, there was not much sun radiation and therefore, the nests did not reach a temperature as high as those nests placed in situ, which could affect embryonic development. In addition, these two studies were carried out at different latitudes. While the Piro beach of our study is near the equator with a latitude of 8.38ºN, the Kochi beach of the study conducted by Kobayashi is located at a latitude further from 33.28ºN. Latitude is the angular distance between the equatorial line (the equator) and a specific point on the Earth. This has an enormous influence on the climate and the temperature of the zone, which may also have caused differences in the results. The angle of solar rays incidence determines the amount of heat a surface receives. The places that are farthest from the equatorial line get less sunlight than those that are closer to the equator. According to this, Piro beach should have had higher temperatures, but the results show higher temperatures in the study carried out in Japan. (Kobayashi et al., 2017). However, the same that happened with emergency success goes for hatching success. It is also higher in the sun, but with a slighter difference that does not even reach the 10%.

The success rate of hatching is 1% greater or more than emergency success, which means that about 1% of hatchlings that managed to hatch, did not manage to leave by themselves or died once they hatched. In addition, infertile eggs can negatively affect the viability of the rest of the eggs as they are often decomposed and colonized by microorganisms that can invade the eggs and therefore affect the success of nests (GETM-IUCN, 2000). This is clearly reflected in our results, since for both areas, the success of hatching exceeds the one for emergence. In the sun, the percentage is surpassed by only a 5%. That is to say that 5% of turtles that hatched from the egg did not manage to emerge whereas in the shade this percentage was exceeded by 7.5%. This means that in the shade the turtles that had hatched from the eggs had a more difficult time trying to reach the surface without success.

This could be related to the compaction of the sand. After the heavy rainy storms, in the shade zone the humidity increased more than in the sun zone, because in the latter the sand dried faster. In the shade, as the sand remains wet, its weight increases and it makes it more compact. This makes it difficult for the turtles to reach the surface of the nest. It could also be related to the fact that the turtles in the sun have more physical strength than the ones in the shade and that they manage to get there by themselves. In addition, a compaction of the sand causes a decrease in oxygen, which can trigger deformities and difficulty of intuitive exit of the nest infants by themselves (Didiher Chacón, Juan Sanchez, 2009).

Overall, it could be said that with our study it is seen that the hatcheries do not negatively affect the survival of the species. It has also been proved that there is a higher survival in the sun zone, since a greater hatching success is directly related to a greater gathering of the population.

Shading is an effective way to decrease the temperature of nests and not to contribute to the creation of species of the same sex (monosexing the species). However, it is not always effective. In many studies, hatching success is greater in shady areas than in sunny areas (Robles & Vega, 2007). For example, the study carried out by Hasbún et al. (1997) in El Salvador on the species *L.olivacea* the survival of nests was better in a total shady area compared with another without shade. This could be the result since the temperatures in the sun area during the study were approximately 34.4ºC, higher than those of our sun zone. It could be these elevated temperatures the cause of the lowest hatching rates in the sun.

Nevertheless, a study conducted by Carrasco-Aguilar (2000) in Mexico, with the species *Lepidochelys kempi* the survival of nests with shade was lower (79%) than in the nests without shade (84%) (Dinarzada et al., 2017). The latter result is similar to the one seen in this study. This diversity of results is the reason why more studies are needed on the survival success of species in different areas.

Therefore, the shading strategy proposed in this study to guarantee sexual diversity and with it the survival of the species in a long-term run could also be negatively affecting survival since it shows lower success rates.

On the other hand, it is known that in those studies the manipulation of the way nests and eggs were transferred to the hatchery was not taken into account. Hatching and emergence success can be affected both positively and negatively by this manipulation. The success or failure in the hatching of the nests and the contribution of new organisms to the population depend to a great extent on the manipulation and handling that is given to the eggs during the collection, transport and planting. Studies show that movement is one of the causes of inhibition in embryonic development and mortality (Dinarzada et al., 2017). Therefore, this is a variable that could also be affecting our results.

5.2. Hatching time

As we mentioned earlier, the eggs of these species take between 40 and 60 days to hatch (Kobayashi et al., 2017). A study conducted in the Ostional beach in Costa Rica recorded an interval between 46 and 56 days for these species (Robles & Vega, 2007). In our results, it is observed that in the sun zone both intervals were not exceeded, with an average hatching time of approximately 57-58 days. In contrast, in the shade the range of days was exceeded, since they usually took about 69 days to hatch, being more than 10 days apart from the sun zone.

Therefore, in the shade a slower development of the embryos is seen, since with lower temperatures the incubation periods are longer. According to Hughes & Richard (1974) the incubation period for the Olive ridley turtle is generally 50 days but it can reach up to 70 days or more depending on the characteristics of the sand in which the eggs are placed. Martínez & Páez (2000) determined that for average temperatures between 28.9 ºC and 32.4 ºC, the incubation period decreases with the increase of temperature. Our results also show this relationship.

In Costa Rica, the threshold temperature of the sand seems to be the key in the hatching time of sea turtles (Sandoval, 2008). Therefore, it is normal that we find differences between the two zones, where there is a very significant temperature difference.

Martínez y Páez (2000) determine that for average temperatures between 28.9 ºC and 32.4 ºC, the incubation period decreases (Robles & Vega, 2007). We can see in our study that the temperature in the sun all day long was about 28.9 ° C, which caused a decrease in the incubation time of our nests. While in the shade the maximum temperature of the day was found at night (27.468ºC).

Shorter incubation periods involve less materials to generate the neonate tissue in embryonic development. Thus, incubation in high temperatures tend to produce smaller turtles with less swimming capacity. For example, Booth and Evans (2011) showed that swimming temperatures affect the frenzy swimming performance in the green sea turtle hatchlings (Kobayashi et al., 2017). Therefore, with this variable we see that an increase in temperature could negatively affect the swimming performance, which in turn affects mortality rates. With these results and those previously discussed, we see that even though more individuals are produced in the sun and in a shorter period of time, they could have less survival skills once in the sea due to their physical conditions. On the other hand, the individuals produced in the shade are lower in number, but they have better swimming conditions that boost their survival.

5.3. Stages

The fertility, as shown by hatching success and opening unhatched eggs (Lutz & Musick, 1997). The unhatched eggs were studied to see in what state of embryonic development they were. Depending on the zone where the eggs were, differences were only observed in the first state (where the embryo does not occupy more than 25% of the egg) and in the third one (where the embryo occupies more than half the egg) (Didiher Chacón, Juan Sanchez, 2009). For both states, we see a greater number in the shade.

Incubation requires several months. During this time, the sea turtle embryos grow from a few cells to a fully formed organism. In this process, energy stored in the egg by the female is transformed into embryonic tissue. Successful incubation of the eggs depends on the presence of suitable conditions in the beach sand. Among these conditions are temperature, humidity or water potential, salinity, and levels of respiratory gases. The microclimate is dynamic and changes with the state of biological activity in the clutch and in the beach. Unfortunately, we know very little about the microclimate of sea turtle eggs during incubation. We know even less about how the physical characteristics of the beach influence the microclimate (Lutz & Musick, 1997). That is why we can not say much about the different stages of embryonic development and about the differences that exist in the two zones.

Development begins immediately following fertilization. Once the yolk has been coated with albumen, the inner shell membrane begins to form. However, the whole shell is not fully formed until at least the seventh day of the following ovulation. The first external sign to see if development is progressing is the emergence of a spot on the uppermost part of the egg. This means that the embryo is situated just beneath the shell. After 55 or more days of incubation, it is difficult to determine whether an egg contains a fertilized embryo that died before being born. Fortunately, the content of unhatched eggs can often be categorized by the presence of blood or by a recognizable embryo at some stage of development. We identified this phase as state 1 where a point was already observed when the eggs were opened (Lutz & Musick, 1997).

The separation of two zones in the hatchery was used as a tool to ensure the diversification of sexes in the species. It is observed in our results that the shade is negatively affecting the success of hatching and emergence. It also affects the number of unhatched eggs in different states of development since it was always greater in the shade. These results suggest a lower survival success of the species in the shade. Therefore, it is important to know at what stage of embryonic development the determination of sex takes place. If this determination occurs before the two stages of development (1 and 3) where in the shade we see a higher percentage of eggs in this stage, letting the sun inside the shade area by opening mesh could be proposed as a solution once sexual determination has taken place.

Nevertheless, different studies show that the sensitive period for sex determination appears to occur around the middle third of incubation. Therefore, the sexual determination occurs when the embryo is in a State 3 or 4 (Lutz & Musick, 1997). For this reason, the solution proposed above would not be viable, since to ensure sexual differentiation the shaded area should be covered until approximately the end of embryonic development which correspond to state 4 and we have no significant differences between sun and shade in this point.

5.4. Effect of temperature change in nests

As a strategy to conserve the species, hatcheries are proposed. It must be borne in mind, that the use of an area where nests were planted in the same conditions could alter the development and survival of the species. Since temperature is an important factor in the development and determination of sex, one of the most crucial points that needs to be taken into account is the way in which nests are handled since their temperature could be affected. Therefore, the implementation of a shaded area was used to avoid monosexing the species. Our results show that the survival of the species can also be negatively influenced. As other studies with the species *Dermochelys coriácea* show, the incubation temperature affects the development of eggs and the emergence of the hatchlings, which agrees with the present study (Dinarzada et al., 2017).

Sea turtle embryos are significantly affected by incubation conditions, especially temperature since it has a strong influence on the rate of development and success of embryos. The incubation temperature influence various parameters of the turtles. Those parameters are the population and individual attributes, the maturation of the eggs, the duration of incubation, the proportion of sexes, the duration of the breeding season and the embryonic survival (Robles & Vega, 2007). Elevated temperatures (higher than 34 ° C) reduce hatching and emergence success rates and increase embryo mortality from early incubation and retardation (Dinarzada et al., 2017). Thus, the temperature monitoring is crucial to understand the incubation environment and if the relocation of the eggs is a good conservation option (GETM-IUCN, 2000).

Laboratory and field studies have shown that sea turtle eggs rarely hatch if they are incubated at constant temperatures <24 ° C or> 35 ° C for a prolonged period. The incubation temperature at which the sex ratio resulting in the clutch is 1: 1, is known as pivotal temperature (approximately 29.3 ° C) (Sandoval, 2008).

To investigate the impact the sun and shade had on our different variables, we assumed that the temperatures at 29,3ºC produced 50% females and 50% males. Temperatures below 27.8◦C produced only males and above 30.8ºC produced only females. The proportion of females increases linearly between 27.8ºC and 30.8ºC (Fuentes & Porter, 2013). As expected, our results showed differences in sex determination according to the zone where the eggs were.

In the sun area temperatures around 29º (pivotal temperature) were found, where we expected to have the same number of females and males. In contrast, the temperature was lower than 27.8ºC in the shade area, which indicated that in the sun zone only males would be produced. All the nests showed a regular variation of temperature with daily fluctuations within a range of 0.3ºC to 1.4ºC (Sandoval, 2008).

These temperature data also support that both survival and sex variation will be more positive in the sun than in the shadow. That means that hatcheries without a shadow area, would be more beneficial at that moment. The current environmental situation of our planet, the climate change, is causing an increase in the temperatures. During our study, a minimum change of the temperature would affect the sand, and therefore the nests. High incubation temperatures would only produce offspring females, thus would lead the species to extinction. Despite the fact that the shadow seems negative for the species in short term, in the long term it is beneficial to sacrifice a few nests with less survival success, to ensure the offspring of male individuals.

If nesting distributions do not change or if they cannot adapt to changing environmental conditions, oviparous species will be threatened by global warming. In addition, their life cycle and reproductive development would also be affected by the probable changes in global temperature, sea level rise, rainfall and increased intensity of hurricanes and storms in tropical areas due to climate change (Dinarzada et al., 2017).

The United Nations Framework Convention on Climate Change (UNFCCC) states that climate change is one of the greatest threats nowadays. The Earth's climate system (atmosphere-oceans-cryosphere) is changing, and will continue to do so at an unprecedented pace in recent human history (GETM-IUCN, 2000).

The global average on the surface of the planet increased 1 ° C between 1880 and 2015. However, the increment is not homogeneous since it is higher in the continents and in the poles (IPCC, 2014). Nevertheless, even though the tropics is the region in the world that emits less greenhouse gases, they could suffer the same way the significant consequences of the impact of climate change on the population, ecosystems and economic activities. The region is highly vulnerable to extreme weather events, increased temperatures, changes in precipitation patterns, reduction of the cryosphere, sea level rise, droughts, floods and hurricanes (GETM-IUCN, 2000).

The last three decades have been successively the warmest since 1850. The temperature on the surface of the oceans rose 0.11 ° C per decade between 1971 and 2010. The IPCC foresaw that the absence of new measures to reduce emissions would probably cause global rise in temperatures that would reach between 3.7 and 4.8 ° C at the end of the century in relation to 1850-1900 (IPCC, 2014).

Specifically, in Central America the temperature has increased between 0.7 and 1ºC since 1970 but rains have decreased one millimeter per day since 1950. In a future scenario, these and other impacts could increase because the temperature is expected to increase between 1.6ºC and 4ºC for 2100. On the other hand, the rains could decrease up to 22% (IPCC, 2014).The objective is to mitigate climate change, so that it only reaches an increase of 1.5ºC. Therefore, if currently in Costa Rica the average annual temperature is between 21 and 27º ("Datos tablas y gráficos mensual y anual las condiciones climáticas en Puntarenas Costa Rica.," n.d.), it is expected that it could reach temperatures of more than 30ºC in the warmest areas of the country such as the Pacific and as reflected in our temperature data, in our study area (Estad & Ambientales, 2017)

Therefore, global warming will cause a higher production of female sea turtles in the future. Consequently, strategies of conservation of low incubation temperatures such as shading the nests, spraying the nests with water or moving the nests to lower depths could be the only way to prevent the extinction of sea turtle population (Laloë et al., 2016). However, the effectiveness of several conservation strategies to mitigate potential threats has not been known yet (Dinarzada et al., 2017).

6. Conclusions

In conclusion, the present study shows that there are significant differences depending on the treatment applied (sun or shade) to the nest of the Olive ridley sea turtle. Hatchlings from the sun had higher survival than those in the shade. It was revealed that lower incubation temperatures negatively affected the emergence and hatchling success, and it caused a higher mortality. Moreover, differences in morphology and neonates' measurements are observed. In the sun, more elongated and narrow hatchling were obtained, while in the shade they were smaller and wider. This indicates that the turtles of the shade will be more agile as they will have better terrestrial locomotion to reach the sea easily without being predated before and a better swimming capacity, which will be useful in the ocean to defend themselves from the predators. Then, elevated temperatures tend to produce smaller hatchlings with less swimming and locomotive capacity. Therefore, this also affects the mortality rates.

Consequently, the sun treatment could have a better output, since more hatching is produced, with more survival and in less time. These hatchlings have less survival skills once in the sea due to their physical conditions. However, less hatchlings are produced in the shade, but they have better conditions for survival once out of the nest.

Furthermore, it is expected that climate change will produce an increase in temperature in the area. If the sun area of our study increased as expected, incubation temperatures would be above 30ºC. Consequently, with these temperatures all the hatchlings would be females, which would lead to the end of the species in the future. This is the reason why nowadays the strategy of making a shade treatment is necessary. If the temperature increases in the area, we would need a shady zone where we can control the temperature, in order to cause lower temperatures as those observed in our study, ensuring the production of males. Therefore, the hope of survival of the specie in the future will increase.

The hatchery is a costly maintenance tool to sustain the material, personnel and time involved. The same goes for the shade treatment. Moreover, both are strategies that generally cause a lower survival of the nest. In balance, they have a negative impact. However, if we look at the long term effect, they are strategies that will benefit the species and guarantee its existence in the future.

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