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Official Title of Study:

Nesting, foraging, and movement ecology of the sea turtle populations within the Gandoca-Manzanillo National Wildlife Refuge (REGAMA) and the functional role they play in the oceanic ecosystem, with emphasis on the hawksbill turtle (*Eretmochelys imbricata*)

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Área de Conservación – La Amistad Caribe, Refugio Nacional de Vida Silvestre Gandoca-Manzanillo

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I. SUMMARY

During the nesting season 2020, our project recorded a total of 186 sea turtle nests that were deposited in the Gandoca-Manzanillo National Wildlife Refuge (REGAMA). Of which, 152 were hawksbill turtle, 27 were leatherback turtle, and 7 were green turtle nests.

153 nests were able to incubate undisturbed. The average clutch success of sea turtle nests in REGAMA was 87.3%. Twenty-three nests were poached by humans (18 hawksbill, 5 leatherback) and ten hawksbill nests were depredated by raccoons and skunks.

The average hatching success of nests varied by species but ranged from 51.58 % in leatherbacks to 82.61 % in green turtles. Nests produced a total of 14,279 baby turtles that made their way to the water (12,831 hawksbill hatchlings, 845 leatherback hatchlings, 603 green turtle hatchlings).

A total of 41 nesting females were identified through external flipper tags and we were able to remove 168 kg of plastic from the beach.

II. INTRODUCTION

The Gandoca-Manzanillo National Wildlife Refuge (REGAMA) is well-known for its incredible terrestrial biodiversity and marine biodiversity. Three species of sea turtles use the refuge for nesting and feeding.

In the past, the primary emphasis of efforts to monitor sea turtle activities in REGAMA has focused on the Gandoca beach sector and the leatherback turtle (*Dermochelys coriacea*) (Chacon *et al.* 1996; Chacon Chaverri 1999; Chacón-Chaverri & Eckert 2007). Although, nestings of hawksbill and green turtles have previously been recorded as well (Figgener 2009). Additionally, individuals of the latter two species have been observed foraging in the reefs and seagrass beds within the REGAMA.

This season, we started a scientific conservation project to monitor the activities of sea turtle populations more comprehensively to improve the overall knowledge of sea turtles within REGAMA and protect nesting females and their eggs. The study's general purpose is to collect data for improved conservation management of the different species. We are establishing a new baseline for the nesting activity and are starting a new in-water database to study the foraging activities and movements of sea turtles within Costa Rican waters and their movements between their nesting and foraging areas to identify migratory corridors. In addition, we are also implementing conservation measures to keep nesting females safe and improve the clutch success and hatching success of sea turtle nests within REGAMA. With these measures, we are ultimately raising the number of adult turtle surviving and increasing the number of hatchlings that will be part of the next generation.

The nesting population of leatherback turtles (*Dermochelys coriacea*) on Playa Gandoca was monitored from 1990 to 2011 by another conservation and research project and included the mark and recapture of females and protection of eggs (Chacon *et al.* 1996; Chacón-Chaverri

& Eckert 2007). During these years, the nesting populations of hawksbill turtles (*Eretmochelys imbricata*) and green turtles (*Chelonia mydas*) were also haphazardly monitored (Figgenger 2009). A variety of different studies of the ecology, population biology, physiology, genetics, behaviour, and migration of the REGAMA sea turtles were also conducted by various researchers and students that provided valuable additional insights (Dutton *et al.* 1999; Furler 2005; James, Eckert & Myers 2005; Gautreau 2007; Spanier 2010; Dutton *et al.* 2013; Figgenger *et al.* 2016).

From 2008 to 2011, due to a conflict of interest, different organisations and stakeholders started to monitor the nesting activities of sea turtles on Playa Gandoca. Unfortunately, the beach was divided during those years, and the separate entities did not share their respective data. From 2011 until 2020, no monitoring was carried out, resulting in a data deficiency of more than ten years and a lack of knowledge of the status of the sea turtle populations within REGAMA. The only data during those years are available from a consultancy of a few months in 2018, which reported 60 clutches of hawksbill turtles and 12 clutches of green turtles between June 20 and October 22 (Fonseca *et al.* 2018). It also noted that four clutches were poached, and seven turtles were killed by people. These data provided evidence that Gandoca still constitutes a critical nesting habitat for sea turtles. In particular, Gandoca seems to be a critical nesting habitat for the critically endangered hawksbill turtle. It further demonstrated the need for a project that monitors nesting activities and protects nesting females and their eggs, establishing a new comprehensive baseline of activities and documenting and preventing further population decline due to anthropogenic and natural causes. In addition, it is of great scientific and conservation value to start an in-water program to monitor foraging activities and habitat use of sea turtles inside the waters of REGAMA and beyond.

Hawksbill Turtle

The hawksbill turtle (*Eretmochelys imbricata*) is one of the smallest species of hard-shelled sea turtles (Cheloniidae). Most of the hawksbill's diet consists of sponges. Individuals have a global distribution in tropical and subtropical waters (Spotila, 2004). The hawksbill turtle is considered *critically endangered* by the International Union for Conservation of Nature (IUCN) (Mortimer & Donnelly (IUCN SSC Marine Turtle Specialist Group), 2008). In the Caribbean culture in Costa Rica, the hawksbill turtle is still hunted for its shell (tortoiseshell), which is used to make jewellery, buttons, combs, knives for illegal cockfighting, and frames for reading glasses (Ministerio de Ambiente y Energia Costa Rica, 2018; Miller *et al.* 2019). Members of this species are also killed within REGAMA (Ministerio de Ambiente y Energia Costa Rica 2018; Fonseca *et al.* 2018).

Previously, important hawksbill nesting activities have been observed in various sectors of the REGAMA, including on some small beaches (*playitas*) north of Punta Mona and the main

beach of Gandoca. In addition, REGAMA also harbours an important foraging habitat for this species, as seen with juveniles, sub-adults, and adults using the reefs.

The Leatherback Turtle

The leatherback turtle (*Dermochelys coriacea*) is the largest sea turtle species (300-900 kg) and has the widest distribution among all reptiles. It can be found in waters from New Zealand to the North of the Arctic Circle (Spotila 2004). One of the largest populations in the western Atlantic Ocean is the Costa Rican population, which has a nesting epicentre in the southern Caribbean of Costa Rica and northern Panama. After the species was globally reclassified as globally *vulnerable* to extinction in 2013 by the IUCN (Wallace, Tiwari & Girondot 2013), a new population-level assessment of the Caribbean population revealed that it is still in danger of extinction (*endangered*) today (The Northwest Atlantic Leatherback Working Group 2019). It shall be noted that other subpopulations are classified as *critically endangered*, as these populations have seen rapid declines on their respective nesting beaches in the Indian Ocean and Eastern Pacific (Spotila *et al.* 2000; Reina *et al.* 2002; Santidrián Tomillo *et al.* 2007; Wallace, Tiwari & Girondot 2013).

However, the history of leatherback nesting inside the REGAMA teaches us something important about long-term monitoring and conservation studies. Historically, Playa Gandoca was an index beach for leatherback nesting and harboured 100-200 females and 300-800 clutches per season (Chacon *et al.* 2007). Through long-term monitoring, important characteristics were identified about the nesting ecology of the species, and particularly the population nesting in REGAMA: *e.g.* it could be established that females re-migrate to nest once every two to three years; that each female lays an average of five to seven clutches per season in re-nesting cycles of nine or ten days; and that an average clutch consists of 70-90 eggs with an *in situ* hatching success of 41% in natural nests (Chacon *et al.* 2007). Thanks to the seamless monitoring and mark-recapture approach over decades, females that came to nest without previous markings were confidently classified as neophytes (first-time nesters). Moreover, the annual percentage of recruitment by neophytes was estimated at ~35 % for this population before 2008. This percentage of neophytes was that of a recovering or stable nesting population of sea turtles and provided evidence for the effectiveness of the conservation efforts implemented.

First-time nesting females constitute a new generation and are crucial to the survival of populations, in this case, the survival of the leatherback turtles in REGAMA, as well as for the West Atlantic subpopulation, where nesting populations have been declining (Troeng *et al.* 2004, Sarti *et al.* 2007; Santidrián Tomillo *et al.* 2007). The arrival of neophyte females on the beaches indicated that coordinated conservation efforts between MINAE, researchers, and different projects, as well as the establishment of protected areas such as REGAMA, are successful (Steyermark *et al.* 1996; Reina *et al.* 2002; Dutton *et al.* 2005; Chacón-Chaverri & Eckert 2007; Santidrián Tomillo *et al.* 2007).

The Green Turtle

The green turtle (*Chelonia mydas*) is the largest species of hard-shelled sea turtles (Cheloniidae). Adults are herbivores and have a global distribution in tropical and subtropical waters (Spotila 2004). The green turtle is considered *endangered* by the IUCN (Seminoff & Southwest Fisheries Science Center 2004). In the Caribbean culture in Costa Rica, the meat and eggs of the green turtle are still consumed, and every year hundreds of females die on beaches in Costa Rica for this reason (Costa Rica. Ministry of Environment and Energy 2018). Individuals within REGAMA are no exception, and individuals and their eggs fall victim to the illegal take (Ministry of Environment and Energy Costa Rica 2018).

Green turtles nest only infrequently and in small quantities within REGAMA, and nesting activities have been mainly observed on the main beach in Gandoca. In addition, REGAMA also provides a potentially critically foraging habitat for this species with large sea grass beds used by juveniles, sub-adults, and adults.

In conclusion, our study and monitoring project aims to provide new long-term data on the activities of sea turtle populations in REGAMA consecutively and comprehensively and protect sea turtle nesting populations.

III. GENERAL AND SPECIFIC OBJECTIVES

The general **long-term objectives** of our research and conservation program are:

- 1) Monitoring of nesting, foraging, and movement activities of sea turtles within REGAMA with an emphasis on the hawksbill turtle, providing population metrics as well as necessary information on natural, anthropogenic effects to the Costa Rican government officials (*e.g.* erosion, illegal collection of eggs and killing of individuals, tourism, garbage, domestic animals).
- 2) Understanding the population dynamics of all sea turtle populations using REGAMA and particularly the habitat use and migratory behaviour of the hawksbill turtle to establish connectivity with other populations and identify migratory corridors.
- 3) Establishing a new baseline of data for sea turtle activities in REGAMA (after data collection was discontinued in 2010) and a long-term monitoring project that will collect continuous data, informing management and conservation plans for sea turtles in Costa Rica generally in the southern Caribbean specifically.

- 4) Determine the role of the physiology and behaviour of the hawksbill turtle in its functional niche within the marine ecosystem.
- 5) Maximise the clutch success of nests deposited and increase the production of hatchlings to guarantee the survival of sea turtle populations in future.

Despite sea turtles' importance to the marine ecosystem, little is known about their zoogeography, ecology, behaviour, and physiology. Our integrated research and conservation approach has several **specific objectives** to illuminate the biology and ecology of sea turtles utilising REGAMA and safeguard nesting females and their eggs. The objectives were as follows:

- 1) Estimate the number of individuals within the respective sea turtle populations in REGAMA using a mark-recapture approach with internal (PIT, Avid) and external (metallic, Monel 49 and Inconel 681 type) tags (Eckert & Beggs 2006);
- 2) Estimate the number of clutches laid during nesting seasons by accurately marking and excavating clutches throughout the season;
- 3) Where possible, determine the effects of the physical environmental conditions of the clutches, such as sand temperature and humidity, on hatching success and sex of hatchlings of leatherback, green and hawksbill turtles (*e.g.* incubation temperature) (Bustard & Greenham 1968; Mrosovsky & Yntema 1980; Mortimer 1990; Chan & Liew 1995; Binckley *et al.* 1998);
- 4) Determine clutch success, hatching success of clutches, as well as causes of embryo mortality by excavating clutches after incubation (Mortimer 1990);
- 5) Determine the reproductive output of females and their respective contribution to the population through the numbers of eggs laid and hatchlings hatched;
- 6) Increase the number of successful clutches and the number of hatchlings by relocating clutches to safer sites (Wyneken *et al.* 1988; Eckert & Eckert 1990);
- 7) Identify the areas of high foraging activities for sea turtles inside REGAMA through transects and captures of turtles in water;
- 8) Characterise the trophic niche of the hawksbill turtle within the REGAMA, including the identification of prey species that constitute part of the diet through observations and stable isotope analysis;
- 9) Characterise the habitat use of hawksbill turtles during the nesting season and after during their post-nesting migration using satellite tracking;
- 10) Establish connectivity between hawksbill nesting and foraging areas in the Greater Caribbean region through satellite tracking to identify important migratory corridors where we can focus conservation efforts in future.

This critical information on the ecology, physiology and behaviour of sea turtles in REGAMA helps us determine their functional role in the oceanic ecosystem. This information is essential for the effective protection and restorations of these endangered species.

IV. DURATION OF THE RESEARCH PROJECT

This final report summarises the data of the nesting season during the year 2020 in Gandoca and describes our seminal monitoring efforts of foraging areas within the REGAMA. However, the scientific monitoring project and included conservation efforts are planned for the long term.

The exact dates of the data collection are June 22 to December 31, 2020. Still, we included data for May provided by the Gandoca Integral Development Association (ADIG) and the MINAE park rangers stationed in the Gandoca sector of REGAMA. In addition, we conducted a track count when we first entered the project site on June 22, 2020, to identify nests laid in the months leading up to June to include that data in the report.

The monitoring efforts started later than planned due to Covid-19 and the government shutdown.

While writing this report, there are still nests on the beach incubating, with estimated hatch dates of January/February 2021.

V. MATERIALS Y METHODS

IV.A. STUDY AREA

The *Gandoca-Manzanillo National Wildlife Refuge* (REGAMA) (Figure 1) is part of the *La Amistad Caribe Conservation Area*. It is located on the southern Caribbean coast of Costa Rica in the district of Sixaola, belonging to the canton of Talamanca in the province of Limón. Playa Gandoca (9 ° 859.9720N, 82 ° 860.5300W) is located inside the REGAMA. The beach has an extension of 8.85 km, with ~ 7.2 km of suitable sea turtle nesting habit, from Punta Mona in the North to the mouth of the Sixaola River. Small beaches of 100 to 300 meters, called *playitas*, located north of Punta Mona, also constitute important sea turtle nesting habitat, particularly for the hawksbill turtle (Figure 2).

REGAMA also harbours some of the last intact coral reefs on the Caribbean coast (Figure 4) (Cortés Núñez 1992) and some seagrass meadows near Punta Mona and the town of Manzanillo (Figure 3) (Krupp, Cortes & Wolff 2009) in which sighting of hawksbill turtles are common and green turtles have also been observed.

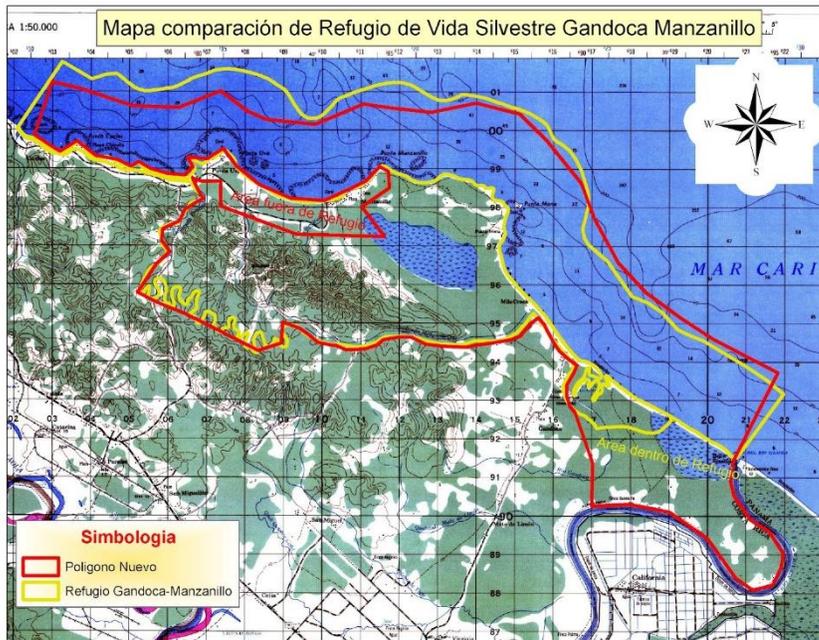


Figure 1. Map of the Project site the Gandoca-Manzanillo National Wildlife Refuge (REGAMA), the project site.

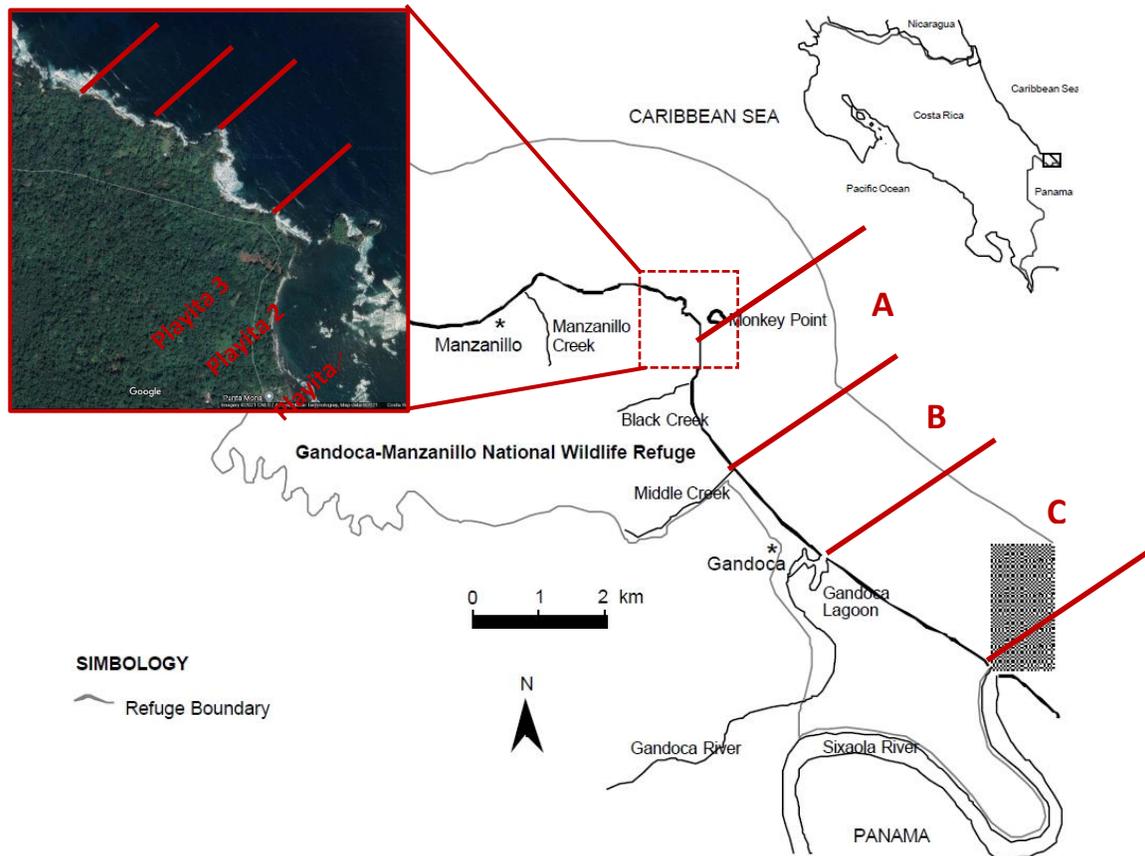


Figure 2. Specific study site for the nesting activities of sea turtles within REGAMA. Shown are the areas that were monitored during the nestings season: three sectors on the main beach (A-C), which divide the otherwise large area, as well as three small playitas close to Punta Mona.

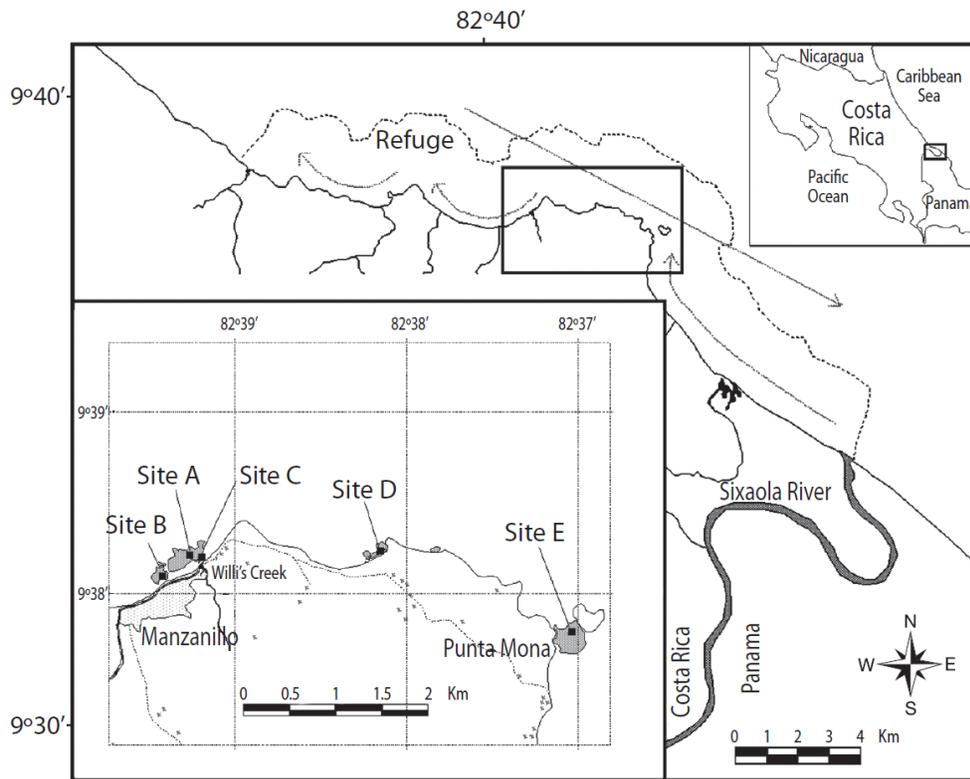


Figure 3. Map of known sites with sea grass beds inside of REGAMA (Krupp et al. 2009)

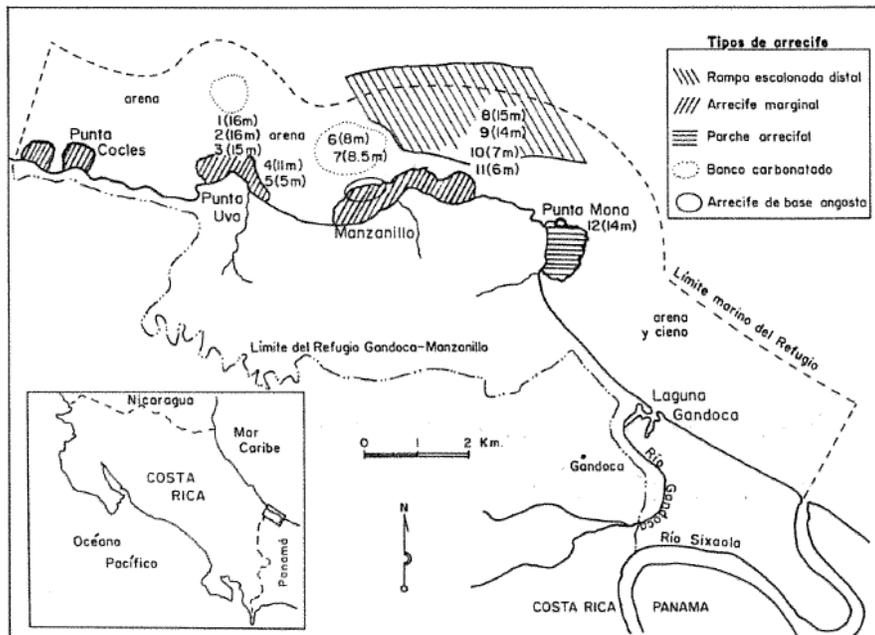


Figure 4. Map of coral reefs inside REGAMA (Cortes Núñez 1992)

IV.B. METHODOLOGY

Our research follows the protocols the sea turtle monitoring techniques detailed in the MINAE / SINAC manual "*R-055-2007 SINAC. Manual for the management and conservation of sea turtles in Costa Rica*" (Chacón *et al.* 2007) and the IUCN manual "*Research and Management Techniques for the Conservation of Sea Turtles*" (Eckert *et al.* 1999). The general handling of turtles and their eggs follows these same protocols and is only done with latex gloves to prevent the spread of pathogens.

We carried out the following activities, described in detail in the sections that follow:

- Preparation of the beach for monitoring
- Nightly patrols and daytime surveys of nesting beaches inside the REGAMA
- Boat surveys inside the REGAMA
- Marking of transects in foraging areas within the REGAMA
- Measurement and data collection of nesting females and foraging individuals
- Marking of nesting females and foraging individuals using internal and external tags
- Relocation of clutches to safer areas of the beach
- Morphometric data collection of neonates
- Excavation of clutches after incubation
- Collection of tissue samples (epidermis and carapace) of hawksbill turtles for stable isotope analysis
- Attachment of satellite transmitters to hawksbill turtles, nesting females and foraging individuals (including the use of white light to carry out this process at night)

IV.B.1 Monitoring of Nesting Activities

IV.B.1.i Preparation of the beach

We divided the main beach into three sectors (Figure 2) to facilitate data collection on nesting locations of females and to delineate nightly patrols during the season. Sector A extends from Punta Mona to Middle Creek (approx. 1.95 km), sector B extends from Middle Creek to the Gandoca Lagoon (approx. 2.85 km), and sector C extends from the Lagoon to the mouth of the Sixaola river (approx. 2.9 km). Within the sectors, we divided the beach into 50 m transects following a line parallel to the sea (Figure 2). We marked each 50 m transect with a wooden marker (*mojon*), painted it with a number in black paint on a white background, and further marked it with two pieces of reflector tape to increase visibility. We started with the number "1" in the North and continued the count until "89" toward the South (starting from Punta Mona to the Gandoca Lagoon). Existing markers from previous seasons were erased or repainted, depending on the condition we found them.

We also carried out beach cleanings to facilitate the nesting of females, removing large driftwood and plastic debris. We continued the cleanups weekly throughout the nesting season and collected data on the found and removed non-organic debris.

IV.B.1.ii Night Patrols

From June 22 to October 31, we conducted nightly patrols on Gandoca beach and the *playitas* to monitor nesting females and hatching clutches. Each patrol was led by a research assistant trained in the appropriate protocols to collect data, relocate eggs, and proper records of nesting events. Red LED flashlights were used during nightly patrols while handling sea turtles, measuring and tagging females, handling eggs, and handling hatchlings. The only exception to the red light rule was during the attachment of satellite transmitters (see V.B.2.IX). The assistants who carried out the patrols were all authorised by MINAE and included in the research permit.

IV.B.1.iii Daytime Censuses

We carried out daytime surveys in the early morning on mornings after nights where we couldn't conduct nightly patrols (e.g. thunderstorms or inaccessibility of certain areas) or when we expected nests to hatch. Our goal was to record all nesting events and collect data on hatched nests. In addition, we used morning surveys to check the status of new natural and relocated clutches, recording all clutches stolen or predated or lost for other reasons.

IV.B.1.iv Registration and Marking of Sea Turtles

Data collection started after a female was done nesting. All nesting females found were identified by external markings, newly or previously. In the case of the leatherback turtle, all the females seen already had tags from other projects. We tagged adult female hawksbill and green turtles with an *Inconel 691* external metal tag on the trailing edge of their right front flipper (Figure 5.B) (Eckert & Beggs 2006) and on the left rear flipper after laying their eggs (Figure 5.A, according to the recommendation of Heidemeyer *et al.* (2018)). We measured the curved carapace length and width of each turtle's carapace, registered each clutch, and counted the number of eggs inside whenever possible (Steyermark *et al.* 1996).

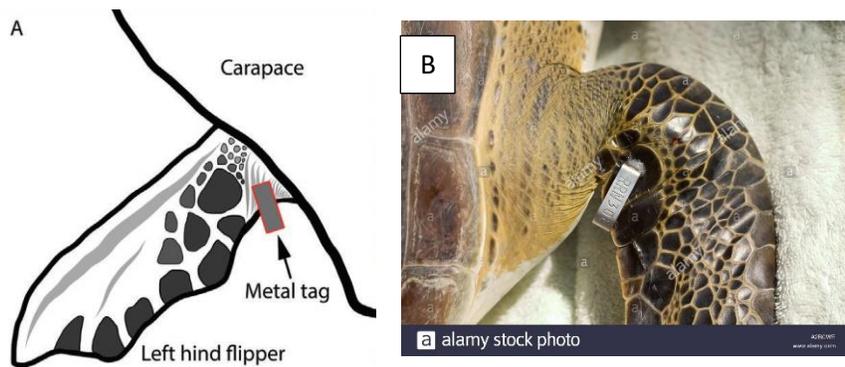


Figure 5. Graphic depiction of the two locations on flippers where external tags are attached in hard-shelled sea turtles. A. Application site in rear flipper (Heidemeyer et al. 2018); B. Application site on trailing edge of front flipper (Eckert & Beggs 2006).

IV.B.1.vi Nest Registry, Relocation, and Excavation

We determined the location of each clutch in relation to the section of the beach and its wooden marker (Steyermark *et al.* 1996).

We registered very high tides and erosion by rivers during the season and a higher than usual activity of recreational fishermen who entered from outside the community. These constituted potential threats and contributed to the number of clutches stolen or destroyed by erosion. For this reason, we moved the majority of clutches to safer places on the beach, where they were protected from possible floods and human impacts within 6 hours after they were laid. In this way, we successfully increased the number of clutches that were able to incubate. The temporary collection of these eggs (handling > 45 minutes) followed established international protocols for handling and transporting sea turtle eggs, including the use of gloves when touching eggs.

Approximately one to two days after hatchlings emerged from a clutch, nest excavations were conducted. During the excavation, we removed the entire remaining nest content (shells and unhatched eggs). The unhatched eggs were opened to determine the developmental stage at which they died, and the number of dead and living neonates was counted to estimate hatching and emergence successes. Live hatchlings were released as quickly as possible (usually within an hour after excavation), usually around sunset (or before sunrise, if the exhumation was done during the night).

IV.B.1.vii Skin and Shell Sampling of Hawksbill Turtles

Part of our study hopes to determine the diet and trophic niche of hawksbill turtles in the REGAMA and wider Caribbean with the help of stable isotope analysis of skin and shell samples. The samples were collected after the turtle finished laying her eggs. First, the sampling site was cleaned with an alcohol swab, and a sample of the skin was taken from the epidermis of the shoulder part (Figure 6) with a 6 mm biopsy punch. Next, a shell sample was taken from the second costal scale in the thickest part of the carapace (Lopez-Castro, Bjorndal & Bolten 2014) with a 6 mm biopsy punch (Figure 7). These samples are stored in cryovials and preserved in alcohol (EtOH 97%). The protocol for sampling, storage of samples, and analysis of isotopes follows the protocols detailed in the manual written by Reich and Seminoff (2010). The samples will either be analysed at the stable isotope laboratory of the Smithsonian Tropical Research Institute in Panama or the newly opened stable isotope laboratory at the Universidad de Costa Rica.

This season, only one skin and shell biopsies of one hawksbill turtle were taken for stable isotope analysis (Figure 6 and 7).

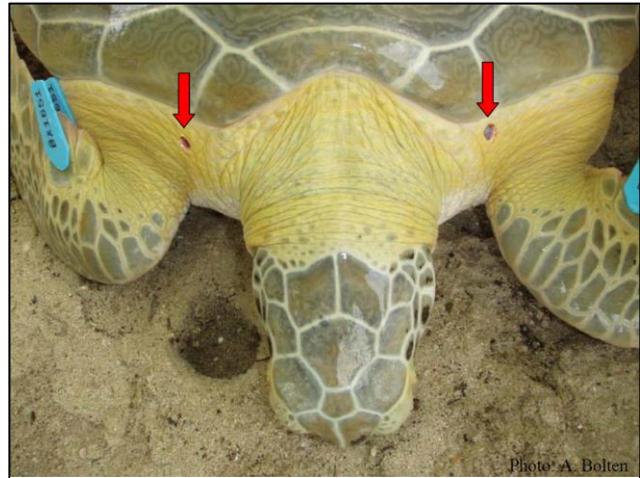


Figure 6. Sampling site for epidermis samples epidermis in hard shelled sea turtles (Reich & Seminoff 2010)

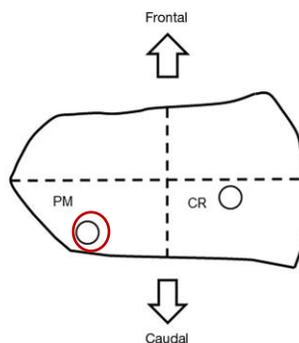
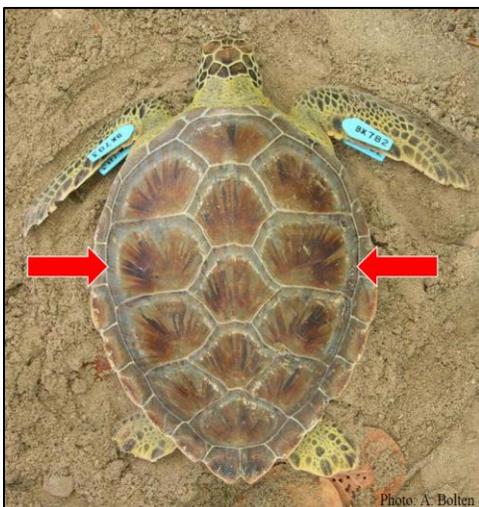


Figure 7. Sampling site for carapace samples in hardshelled sea turtles (Lopez-Castro et al. 2014, Reich & Seminoff 2010)

V. B.1.viii Stable Isotope Analysis

Quantifying resource use over time among individuals within natural populations is challenging. However, recent advances in biogeochemical approaches have provided the possibility of characterising the trophic niche of populations and individuals by using stable isotope analysis (SIA) of tissues to quantify the isotopic niche and use it as a proxy for the trophic niche (Michener & Lajtha 2007). Stable isotopes are intrinsic markers assimilated through food, water, and gas entering the body (Rubenstein & Hobson 2004). The two stable isotopes most used for trophic ecology studies are stable carbon (^{13}C) and stable nitrogen (^{15}N). The stable isotope composition of a consumer is determined by the ratio of light to heavy isotopes (*e.g.*, $^{12}\text{C}:^{13}\text{C}$ written as $\delta^{13}\text{C}$) from their dietary sources (Hobson 1999). Due to the selectivity of the heavier isotopes during metabolic processes, animal tissues tend to be enriched relative to their diet by a discrimination factor of 0 to 1 ‰ for $\delta^{13}\text{C}$ (DeNiro & Epstein 1978) and 3 to 4 ‰ for $\delta^{15}\text{N}$ by trophic level (DeNiro & Epstein 1981). Stable isotope analysis uses this predictable source-to-consumer discrimination to make ecological predictions. For example, in the marine environment, stable carbon reflects the isotope ratios of primary producers in a food chain, which in turn indicates the type of habitat in which an organism feeds (DeNiro & Epstein 1978; Hobson 1999; Rubenstein & Hobson 2004). Stable nitrogen indicates the trophic position of an organism within its food chain (DeNiro & Epstein 1981; Hobson 1999; Rubenstein & Hobson 2004). Together, the combination of the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of each individual provides a quantitative isotopic niche, which can be interpreted as the trophic niche of a species or population (Bearhop *et al.* 2004; Semmens *et al.* 2009; Marshall *et al.* 2019).

IV. B.2.IX Attachment of Satellite Transmitters to Hawksbill Turtles

The transmitter placement process is performed after oviposition. Ultrasound imaging is used to determine the reproductive stage by checking the ovaries and oviducts for follicles or eggs that may remain in the reproductive tract. To follow females in their post-nesting migrations, we look for individuals that have finished nesting and no longer have follicles and eggs in their ovaries and oviducts, respectively. To analyse habitat use within the REGAMA and Costa Rican waters, we look for females with follicles and eggs in their ovaries and oviducts, respectively, and are expected to nest again.

This season we used the Telonics SeaTrkr-4370-4 transmitter (Figure 8) (Figgenger, Bernardo & Plotkin 2018). These transmitters are very small and light (210 g and 10.3 cm x 4.5 cm x 3.6 cm). A protocol established by Balazs, Miya and Beavers (1996) and optimised for juveniles and adults by Mansfield *et al.* (2012) was used to install this type of platform transmitter. First, the carapace was cleaned with water and a brush to algae and microorganisms. Next, the remaining fats were removed by washing the shell several times with acetone. The carapace and transmitter were roughed up with sandpaper to improve the attachment of adhesive. A cold-setting epoxy glue (Universal Adhesive PN 8217 from 3M) was used as adhesive with which the transmitter was glued onto the highest and flattest part of the carapace. The turtle was held by hand during the attachment process and until the adhesive was completely cured. After the attachment process, the turtle was released and free to go back to the water. When attaching satellite transmitters at night, we usually use white lights but cover the turtle's eyes with a dark and wet cloth.



Figure 8. Photo of the satellite transmitter SeaTrkr-4370-4, here attached to an olive ridley turtle.

This season, one nesting hawksbill turtle was equipped with a satellite transmitter.

IV.B.2 Monitoring of Foraging Activities

IV.B.2.i Sampling of Foraging Sites

As often as possible, expeditions were carried out in the coastal waters of the Gandoca Manzanillo National Wildlife Refuge, including San Juanillo, to find hawksbill (*E. imbricata*) and green turtles (*C. mydas*) foraging on the reefs, coral reefs, and seagrass meadows.

Counts were carried out through transects at various sites in the marine sector of the REGAMA.

VI. RESULTS

V.1. Nesting Activity

V.1.a. Hawksbill Turtle (*Eretmochelys imbricata*)

Nesting Activity (Temporal and Spatial)

Between June 22 and December 31, we recorded 124 hawksbill nests on the main beach of Gandoca and the three playitas monitored. The last clutch of eggs was laid on November 20, 2020. Before June 22, 28 nests were registered by the Gandoca Integral Development Association (ADIG) and the MINAE park rangers in Gandoca. These 28 nests were confirmed in parts by an initial track count that we conducted on June 22.

We recorded the highest nesting activity between June and September, with a peak in July (n = 43 clutches, Figure 9)

A large part of the clutches (n = 69 clutches) was laid in the main Playita (Figure 2), but the main beach of Gandoca also harboured a significant number (n = 71 clutches, Figure 10).

Compared to the nesting activity over the past 23 years (Figure 11), the number of clutches is much higher than in the years for which we have data. The years with the highest number of clutches previously recorded were 1997, 2009, and 2018, and each year had about 60 clutches. One possible reason why we documented more clutches is our increased monitoring effort. For the first time in the history of Gandoca, we continuously monitored potential nesting beaches throughout the months of the highest nesting activity of hawksbill turtles (July to October). Previous monitoring efforts usually ceased at the end of the leatherback season (June), the main focus of previous sea turtle monitoring projects in Gandoca.

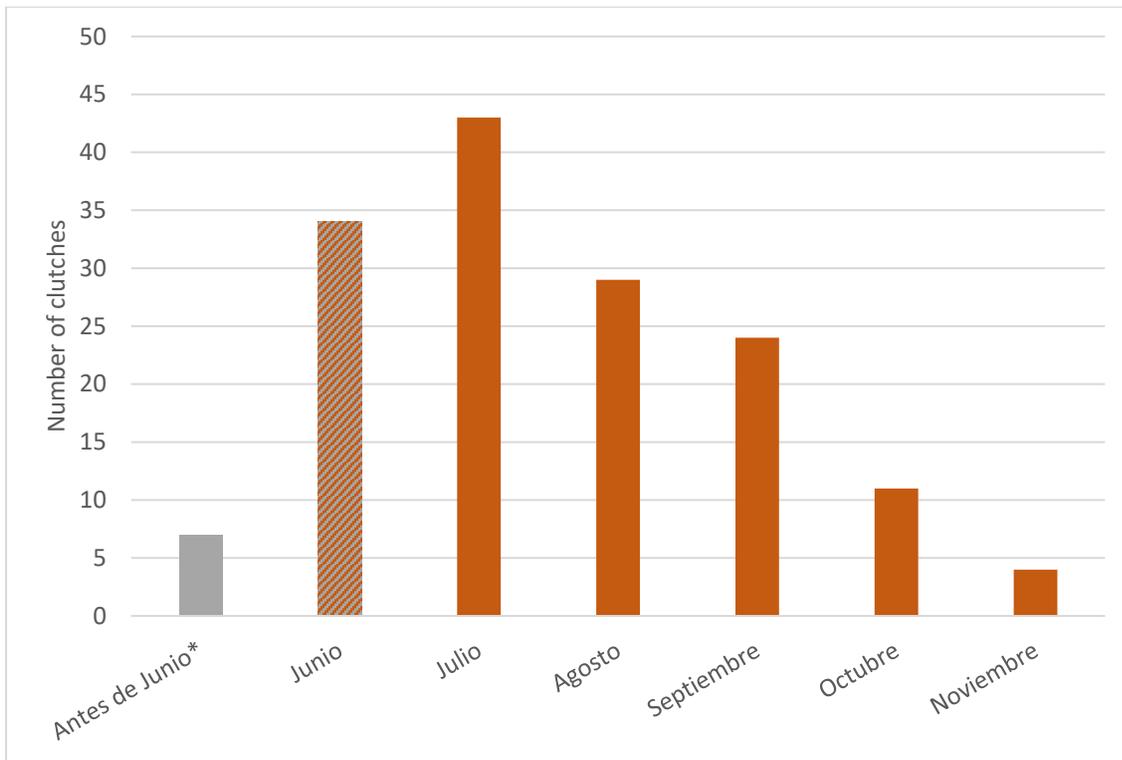


Figure 9. Nesting activity of the hawksbill turtle (*Eretmochelys imbricata*) in the Gandoca-Manzanillo National Wildlife Refuge during the 2020 season. Number of clutches per month. (* Data provided by the Gandoca Integral Development Association and the Gandoca sector park rangers)

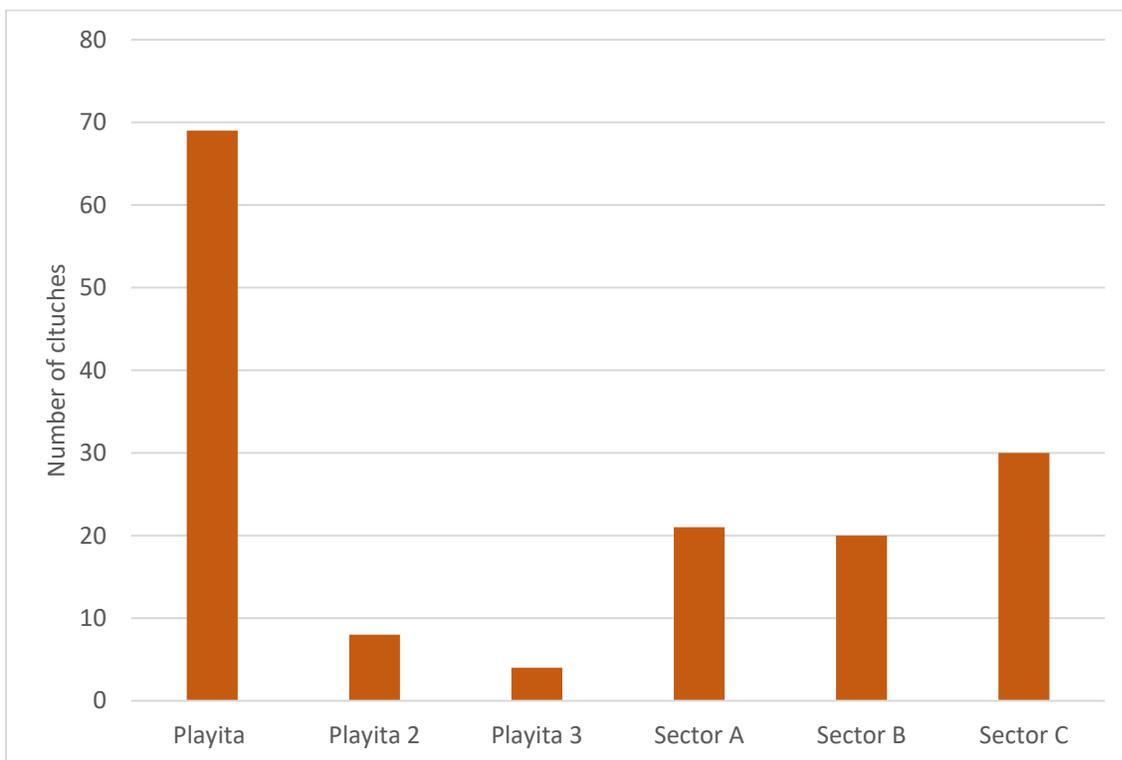


Figure 10. Nesting activity of the hawksbill turtle (*Eretmochelys imbricata*) in the Gandoca-Manzanillo National Wildlife Refuge during the 2020 season. Number of clutches by sector on the beach.

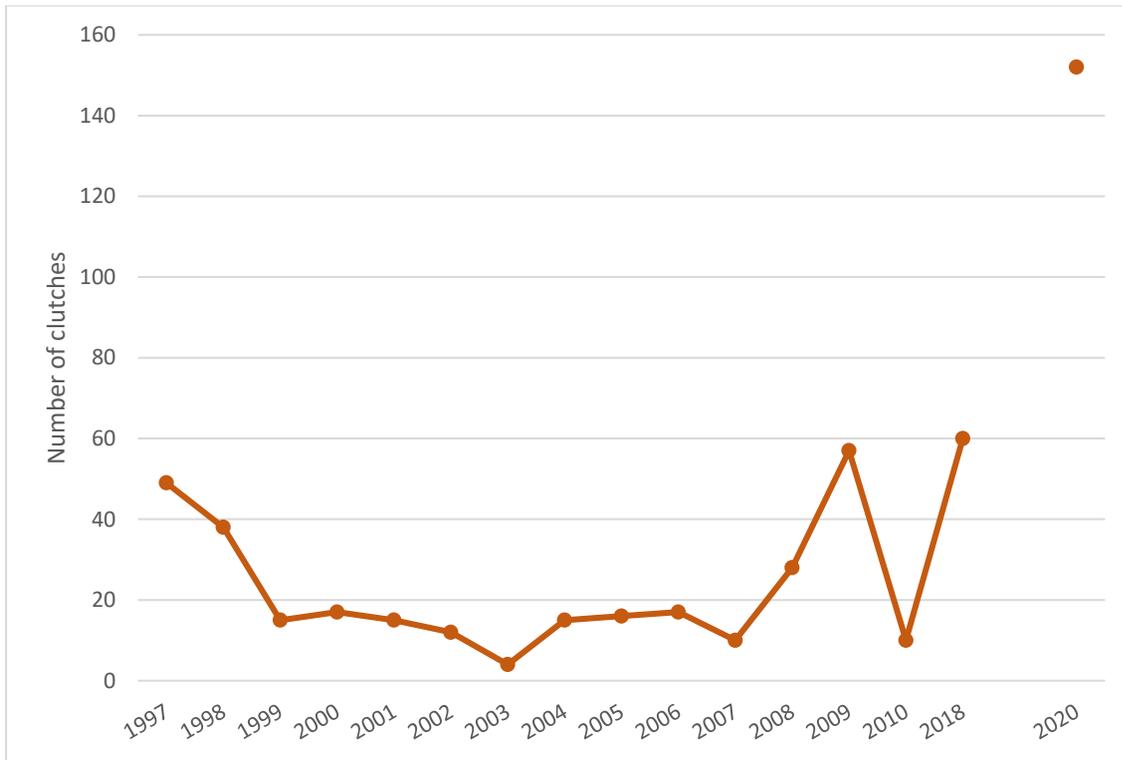


Figure 11. Nesting activity of the hawksbill turtle in REGAMA from 1997-2020. Number of clutches per year. Large data gap from 2010 to 2018.

Clutch Fate

Most of the hawksbill clutches laid (n = 107 clutches) were successfully relocated to safer places on the beach, and none of those clutches was stolen by humans (Figure 12). Seventeen nests remained natural and incubated successfully (Figure 12). However, some of the relocated and natural nests were predated (n = 10 clutches) by raccoons (*Procyon spec.*) and skunks (*Conephus spec.* or *Mephitis spec.*) (Figure 12). In the future, measures must be taken to prevent this type of depredation. In addition, some recently laid nests (n = 18 clutches) were stolen before the research assistants arrived at the site (Figure 12). Most of these clutches were stolen in La Playita (n = 8 clutches) and in sector C near the Gandoca lagoon (n = 8), probably from fishermen who entered at night.

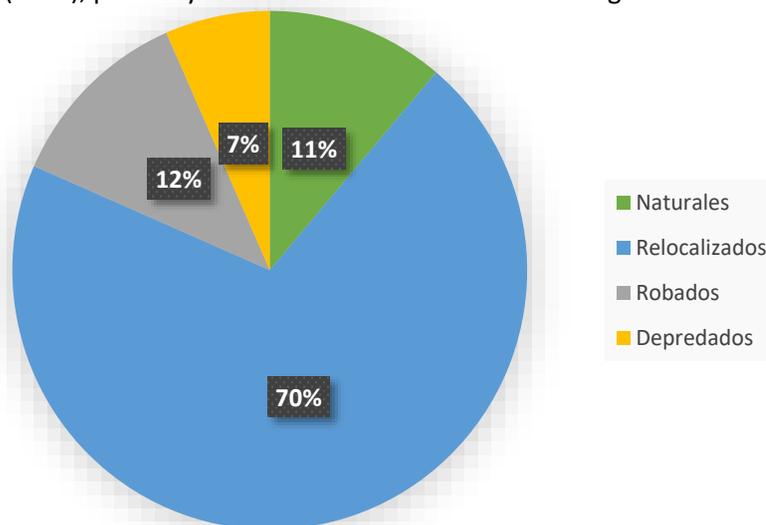


Figure 12. Fate of hawksbill turtle (*Eretmochelys imbricata*) clutches during the 2020 nesting season in the Gandoca-Manzanillo National Wildlife Refuge. Naturales = natural; Relocalizados = Relocated; Robados = Poached; Depredados = Depredated;

Clutch Success and Number of Offspring Produced

The clutch success of hawksbill clutches in 2020 was 80.26% (122 of 152 nests hatched successfully).

The mean incubation period was 62.7 days ($n = 44$, range 50-102, $SD \pm 9.94$). We exhumed a sample of 75 hawksbill clutches. The mean hatching success of the clutches was 68.01% ($n = 75$, range 0-99.46, $SD \pm 30.81$). The clutches with zero hatching success were those left natural and washed away by the tide.

The mean emergence success of the clutches was 54.03% ($n = 75$, range 0-98.53, $SD \pm 35.07$) and is similar to that of other hawksbill populations in the Caribbean, including Tortuguero in Costa Rica (Witzell 1983; Bjorndal *et al.* 1985). In some nests, the young were trapped inside roots inside the nest and could not get out independently. Due to the exhumations that we carry out from 24 hours to 48 hours after most babies hatched, many babies stuck inside the nest were rescued and released.

The mean size of hawksbill hatchlings was 4.12 cm straight carapace length (SCL) ($n = 15$ clutches (150 hatchlings), range 3.51-4.45, $SD \pm 0.249$) and 2.88 cm straight carapace width (SCW) ($n = 15$ clutches (150 hatchlings), range 2.44-3.18, $SD \pm 0.204$). Hawksbill hatchlings weighed an average of 15.07 g ($n = 15$ clutches (150 hatchlings), range 11.04-18.72, $SD \pm 2.16$). These data are similar to those for other populations of hawksbills (Witzell 1983).

Based on the number of clutches laid, clutch success, number of eggs laid per female, and hatching success per average, **we estimate that 12,831 hawksbill hatchlings hatched and emerged successfully** in REGAMA in 2020.

Size of Nesting Population, Mark-Recapture Results, Size of Nesting Females, and Reproductive Output

We identified 35 different nesting females during the season. Two females arrived with external tags from other projects, and 33 were tagged by us. The average number of clutches per female is three to four clutches (Witzell 1983; Spotila 2004), and we estimate that some 43-50 females arrived at REGAMA to nest in the 2020 season.

The re-nesting interval during the season was 14.2 days ($n = 27$, range 13-18, $SD \pm 1.23$). This interval is similar to that observed in other hawksbill populations (Witzell 1983; Bjorndal *et al.* 1985; Kamel & Delcroix 2009)

The mean size of nesting females was 86.3 cm curved carapace length (CCL) ($n = 46$, range 79.4-95.1 cm, $SD \pm 4.31$) and 77.24 cm curved carapace width ($n = 46$, range 69.8-88.8 cm, $SD \pm 4.66$)

Nesting females laid an average of 154.67 eggs per clutch ($n = 113$, range 48-207 eggs, $SD \pm 30.07$) and **produced a total of 23,408 eggs during the 2020 season.**

Table 1. List of individual female hawksbill turtles identified in the Gandoca-Manzanillo National Wildlife Refuge during the 2020 season. Migratory status refers to three categories: females previously tagged in other seasons (remigrant - REM), females tagged in the same season but on another nesting beach (re-nesting - REN), and females tagged for the first time this season by us (recruit - REC).

Individual #	Left Tag	Right Tag	Migratory Status	Place Tagged (year)
1	0032CR	8343	REM	Unknown
2	0120CR	150722	REN	STC-Tortuguero (2020)
3	10934	10933	REC	REGAMA (2020)
4	10936	10937	REC	REGAMA (2020)
5	10940	10941	REC	REGAMA (2020)
6		10942	REC	REGAMA (2020)
7	0005CR	10944	REC	REGAMA (2020)
8	0001CR	0002CR	REC	REGAMA (2020)
9	0003CR	0004CR	REC	REGAMA (2020)
10	0007CR	0008CR	REC	REGAMA (2020)
11	0009CR	0010CR	REC	REGAMA (2020)
12	0011CR	0012CR	REC	REGAMA (2020)
13	0013CR	0014CR	REC	REGAMA (2020)
14	0015CR	0016CR	REC	REGAMA (2020)
15	0027CR	0026CR	REC	REGAMA (2020)
16	0029CR	0028CR	REC	REGAMA (2020)
17	0031CR	0030CR	REC	REGAMA (2020)
18	0035CR	0036CR	REC	REGAMA (2020)
19	0037CR	0038CR	REC	REGAMA (2020)
20	0054CR	0052CR	REC	REGAMA (2020)
21		0055CR	REC	REGAMA (2020)
22	0056CR	0058CR	REC	REGAMA (2020)
23	0059CR	0060CR	REC	REGAMA (2020)
24	0061CR	0062CR	REC	REGAMA (2020)
25	0063CR	0064CR	REC	REGAMA (2020)
26	0066CR	0067CR	REC	REGAMA (2020)
27	0068CR	0069CR	REC	REGAMA (2020)
28		0069CR	REC	REGAMA (2020)
29	0071CR	0072CR	REC	REGAMA (2020)
30	0073CR	0099CR	REC	REGAMA (2020)
31	0101CR	0102CR	REC	REGAMA (2020)
32	0103CR	0104CR	REC	REGAMA (2020)
33	0105CR	0106CR	REC	REGAMA (2020)
34	0107CR	0108CR	REC	REGAMA (2020)
35	0075CR		REC	REGAMA (2020)

Ecological study of trophic niche and habitat use of the hawksbill turtle

During the 2020 season, we attached one satellite transmitter to a hawksbill female. We didn't attach more because of the difficulties bringing more transmitters from the USA to Costa Rica during the pandemic. The tagged female nested on September 23, 2020, in sector C of the main beach of Gandoca. Ultrasound images showed that she still had many follicles in her ovaries, indicating that she was going to nest again.

Skin and shell samples were taken from this female to be used for stable isotope analysis in future. With this analysis, we can evaluate the trophic niche and the diet of the female.

From the GIS data that the tag provided, we observed that the female stayed a few days on Uvita Island in front of Limón after nesting in Gandoca and before swimming into deeper waters (Figure 14). On October 4, 2020, she nested again for the last time in the 2020 season, not in Gandoca as we expected, but in Isla Machuca near Barra Colorado and Tortuguero National Park (Figure 15). She then started travelling North.

While writing this report, the transmitter is still transmitting, and the female is located slightly northeast of Isla Cozumel in Mexico (Figure 16).

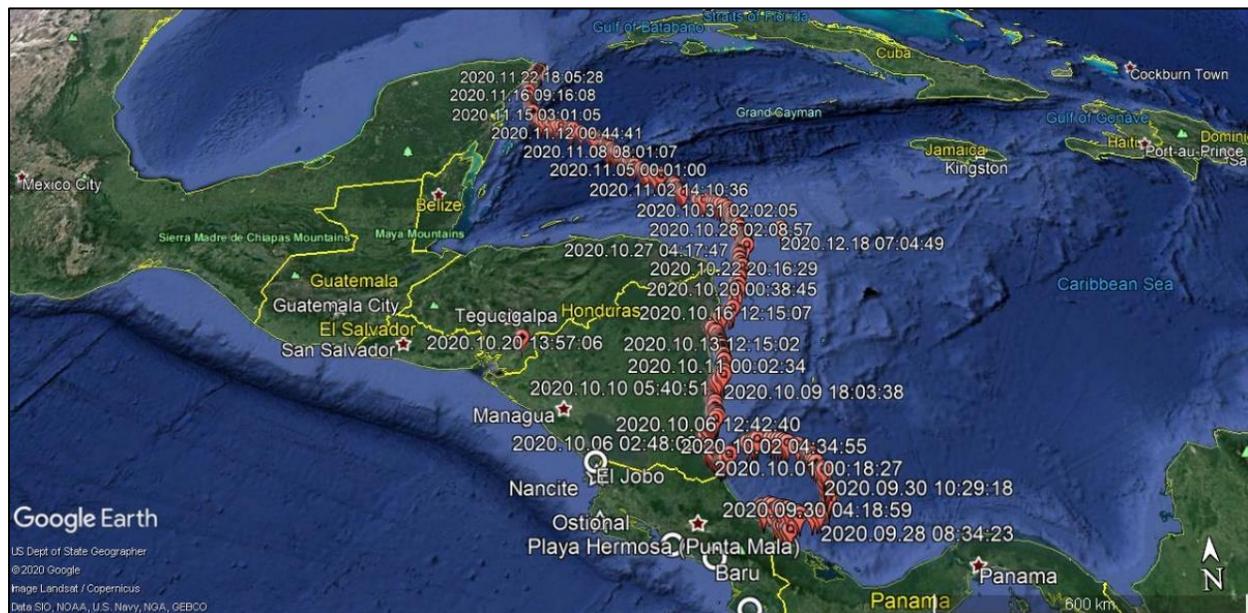


Figure 13. Map of the entire track of the hawksbill female equipped with a satellite transmitter on September 23rd, 2020 in REGAMA. The turtle swam from Costa Rica to the Island of Cozumel in Mexico to forage.

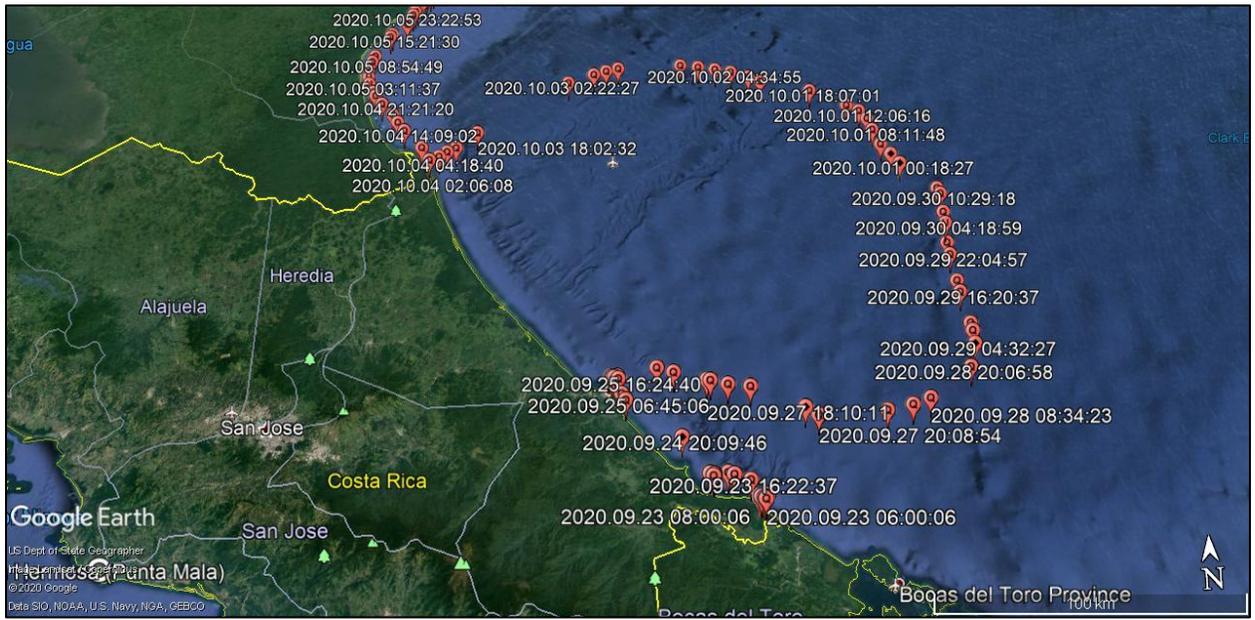


Figure 14. Map of the GPS locations and the track within Costa Rican waters from the hawksbill female equipped with a satellite transmitter on September 23, 2020 in REGAMA.

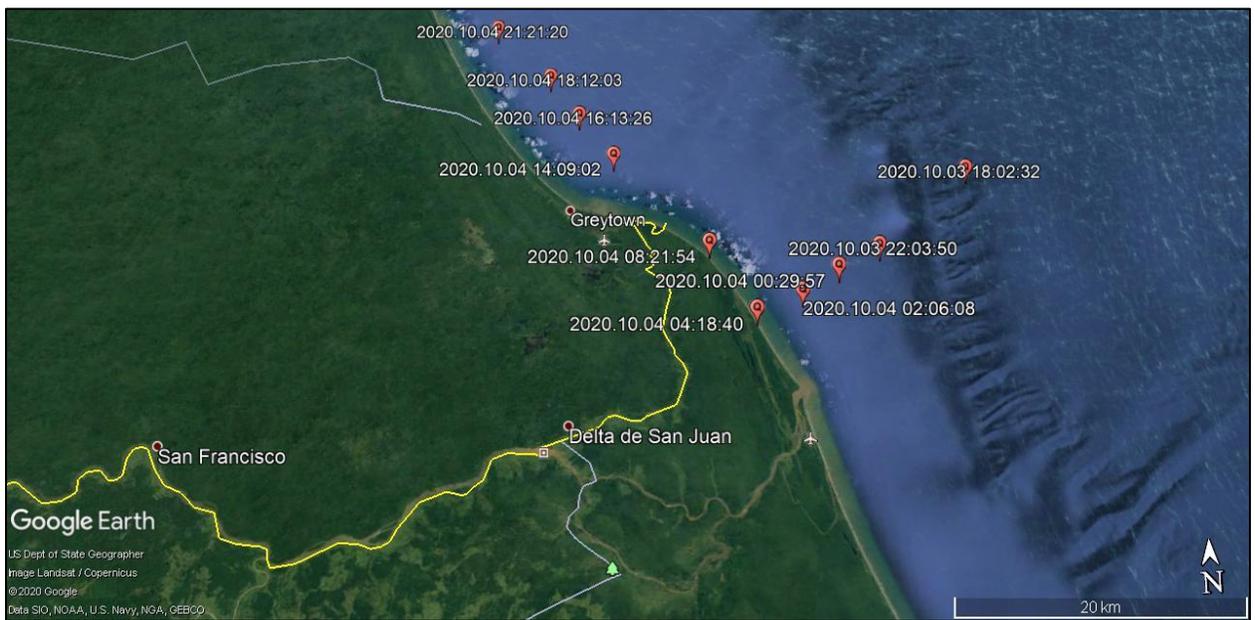


Figure 15. Map of the GPS locations in the North of Costa Rica that are indicating a second nesting event from the hawksbill female equipped with a satellite transmitter on September 23, 2020 in REGAMA.

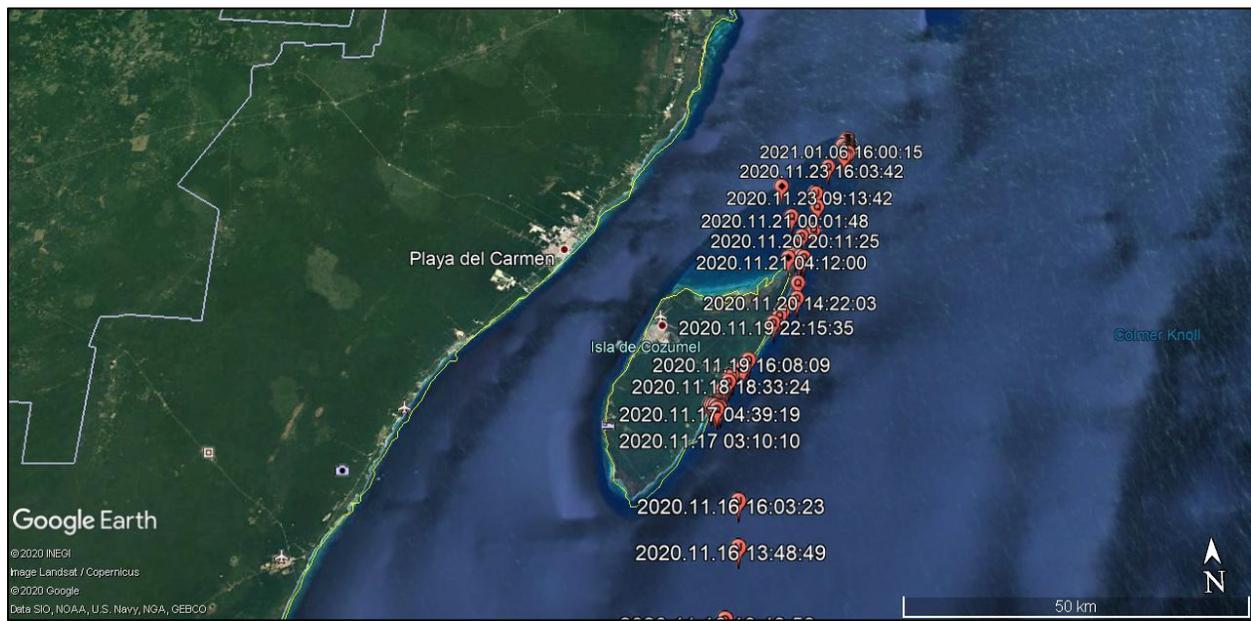


Figure 16. Map of the GPS locations close to the Isla Cozumel in Mexico from the hawksbill female equipped with a satellite transmitter on September 23, 2020 in REGAMA.

V.1.b Leatherback Turtle (*Dermochelys coriacea*)

Nesting Activity (Temporal and Spatial)

Between June 22 and December 31, 2020, 27 leatherback nests were recorded on the main beach of Gandoca. The last clutch of the season was laid on July 12. Before June 22, 24 nests were registered: five by the Gandoca Integral Development Association (ADIG) and the MINAE park rangers in Gandoca, and 19 via an initial track count we conducted on June 22.

The highest nesting activity cannot be analysed because data collection did not begin until June (Figure 17)

A large part of the recorded clutches (n = 12) was laid in sector C of the main beach of Gandoca (Figure 18).

Compared with activity during the last 23 years (Figure 19, Chacón-Chaverri and Eckert (2007)), the number of clutches is much lower than in the years we have data. But we should not forget that our data is incomplete because we started the monitoring at the end of the leatherback season.

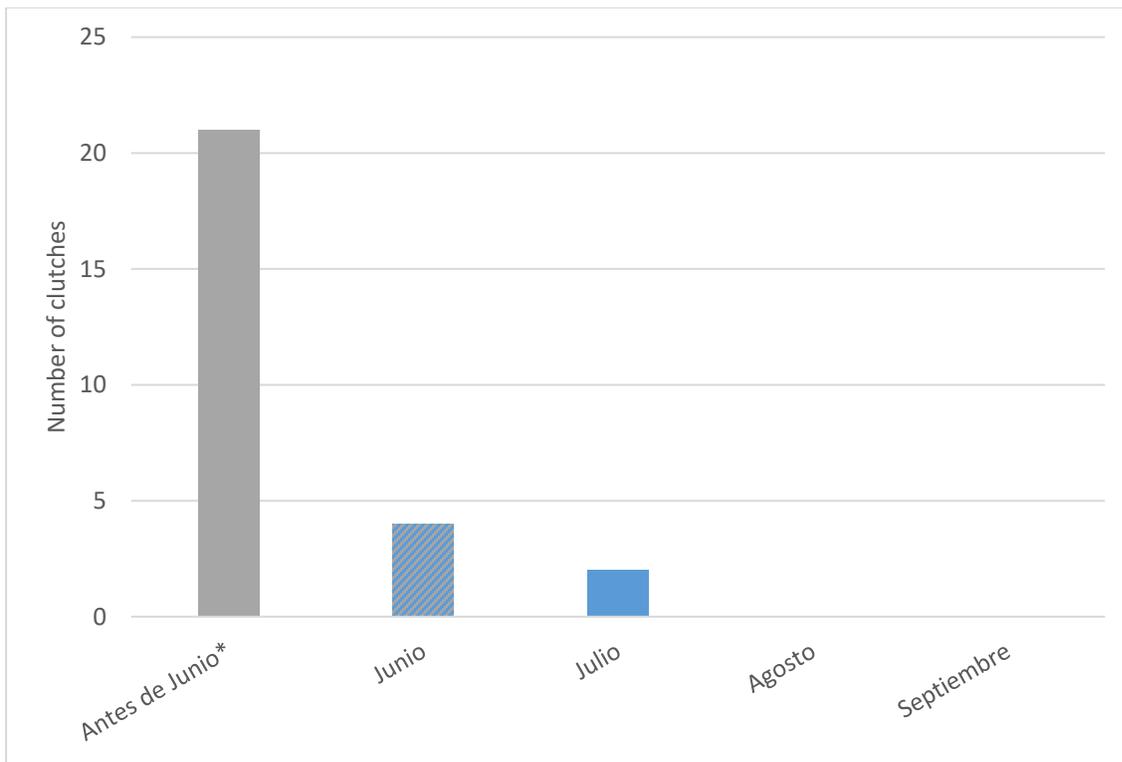


Figure 17. Nesting activity of the leatherback turtle (*Dermochelys coriacea*) in the Gandoca-Manzanillo National Wildlife Refuge during the 2020 season. Number of clutches per month (* Data provided by the Gandoca Integral Development Association and the Gandoca sector park rangers).

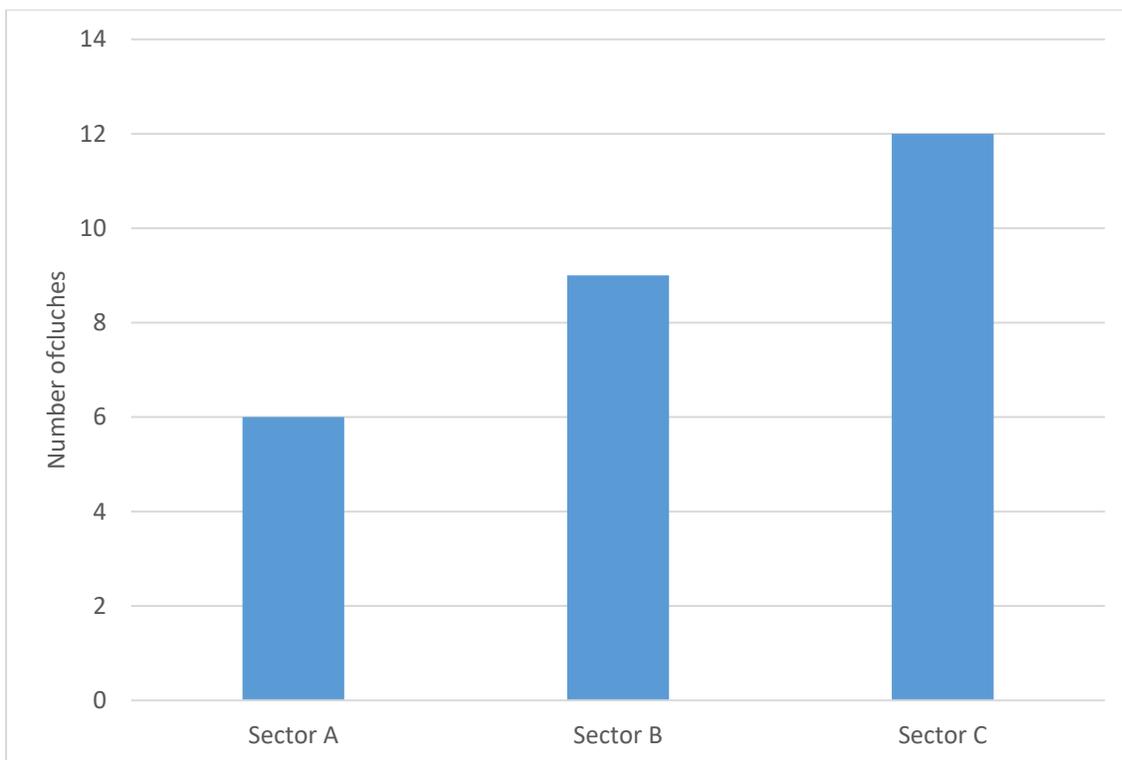


Figure 18. Nesting activity of the leatherback turtle (*Dermochelys coriacea*) in the Gandoca-Manzanillo National Wildlife Refuge during the 2020 season. Number of clutches by sector on the beach.

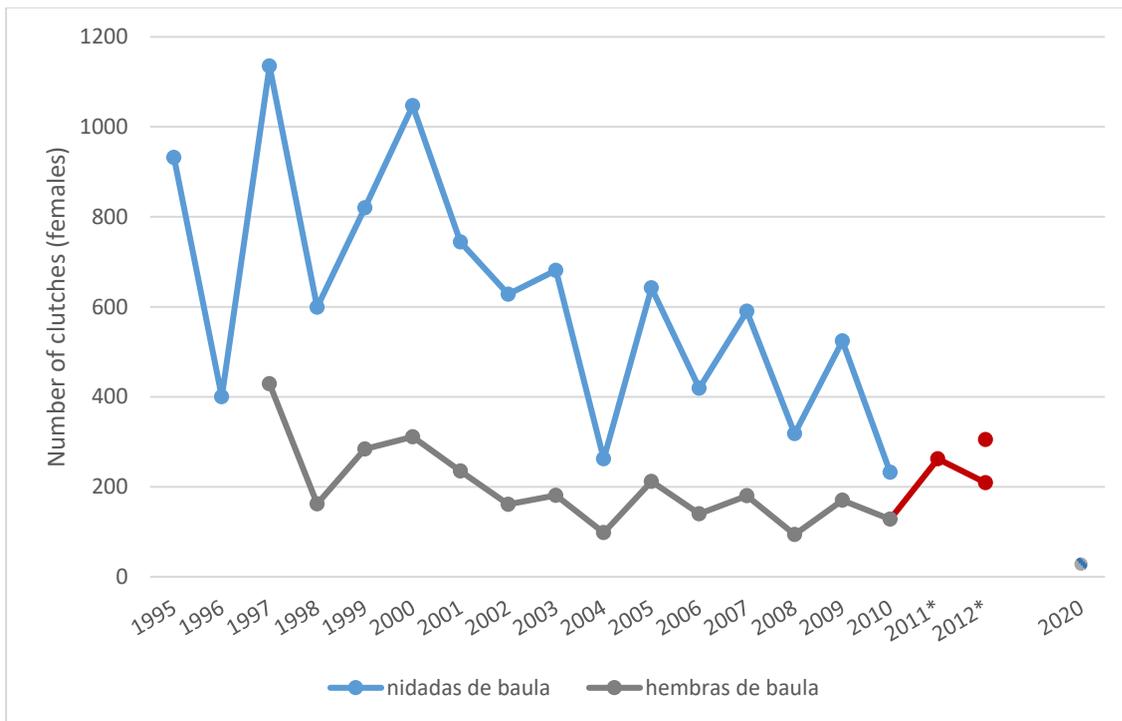


Figure 19. Nesting activity of the leatherback turtle in REGAMA from 1995-2010. Number of clutches per year (nidadas de baula), and number of females per year (hembras de baula). Large data gap from 2010 to 2020. The data from 2011 and 2012 are not very reliable and the data from 2020 are incomplete. Nidadas de baula = leatherback clutches; hembras de baula = leatherback females.

Clutch Fate

Most of the leatherback clutches were laid before June 22 and remained in their natural state (n = 16 clutches) (Figure 20). Six clutches were successfully relocated to safer places on the beach and none of those clutches were stolen by humans (Figure 20). Some of the nests that were laid before June 22 were stolen (n = 5 clutches). Almost all of these clutches were stolen in Sector C, only one was stolen in Sector B. No clutch of the leatherback turtle was preyed upon.

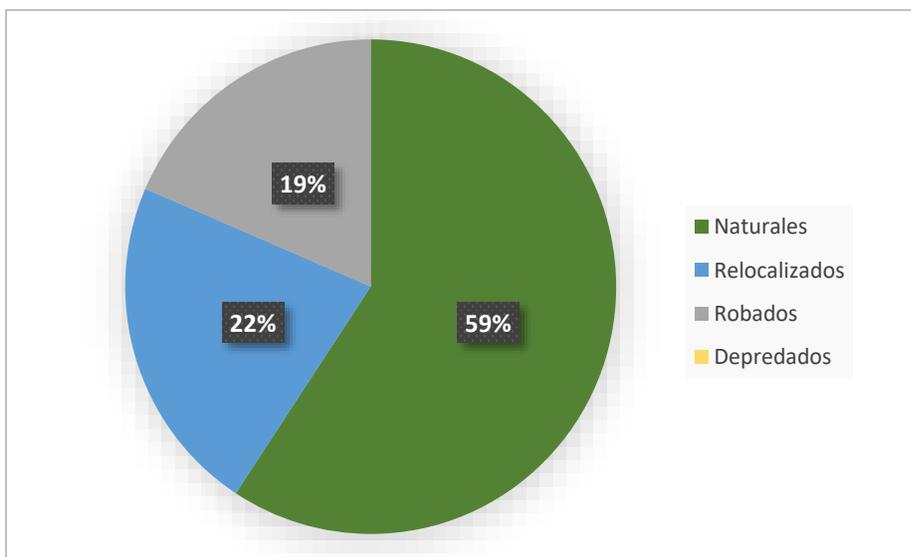


Figure 20. Fate of leatherback turtle (*Dermochelys coriacea*) clutches during the 2020 nesting season in the Gandoca-Manzanillo National Wildlife Refuge. Naturales = natural; Relocalizados = Relocated; Robados = Poached; Depredados = Depredated;

Clutch Success and Number of Offspring Produced

The clutch success of leatherback turtles was 81.48% (22 of 27 nests hatched successfully) in 2020.

The mean incubation period was 59 days (n = 2, range 58-60 days, SD ± 1.41).

We exhumed a sample of 4 leatherback turtle clutches. The mean hatching success of the clutches was 51.58% (n = 4, range 6.56-83.1%, SD ± 35.99). The mean emergence success was 43.23% (n = 4, range 6.56-77.46%, SD ± 31.43). In some nests, the hatchlings were trapped inside the nest and could not get out on their own because of roots. Due to the exhumations that we carry out 24 hours to 48 hours after most of the hatchlings have left the nest, many babies were rescued and released.

Based on the number of clutches laid, clutch success, number of eggs laid per female, and mean hatching success rate, we estimate that at least **845 leatherback hatchlings hatched and emerged successfully in REGAMA during 2020.**

Nesting Population Size, Marking-Recapture Results, Female Size, and Reproductive Output

Three different nesting females were identified during the season. All females came with external tags from other projects. The average number of clutches per female is five to seven, but they are not very faithful to a single nesting site (Spotila 2004; Eckert *et al.* 2012). Therefore, we estimate that at least 5-20 females contributed to the nests in REGAMA during the 2020 season.

The mean size of females was 149 cm curved carapace length (CCL) and 112.8 cm curved carapace width (CCW).

Female leatherbacks laid an average of 74.5 eggs per clutch (n = 6, range 61-91 eggs, SD ± 12.37) and **produced a total of 1,639 eggs during the 2020 season.**

Table 2. List of individual female leatherback turtles identified in the Gandoca-Manzanillo Wildlife Refuge during the 2020 season. Migratory status refers to three categories: females previously tagged in other seasons (remigrant - REM), females tagged in the same season but on other beaches (re-nesting - REN), and females tagged for the first time this season (recruit - REC).

Individual #	Left Tag	Right Tag	Migratory Status	Place Tagged (year)
1	VC6896	VC6897	REM	Unknown
2	VC7857	VC7744	REM	Unknown
3	VC2674	VC2675	REM	Unknown

V.1.c. Green Turtle (*Chelonia mydas*)

Nesting Activity (Temporal and Spatial)

Between June 22 and December 31, 2020, we recorded seven green turtle nests on the main beach of Gandoca and the three playitas we monitored. The first clutch was laid on July 23, and the last clutch was laid on September 30. Before June 22, no green turtle nests were recorded.

We recorded the highest nesting activity in September (n = 5 clutches, Figure 21)

A large part of the clutches (n = 4 clutches) was laid on the main beach of Gandoca, but there were three clutches that were laid on the main *playita* (Figure 22).

Compared to the nesting activity over the past 23 years (Figure 23), the number of clutches is within the range of the years we have data for.

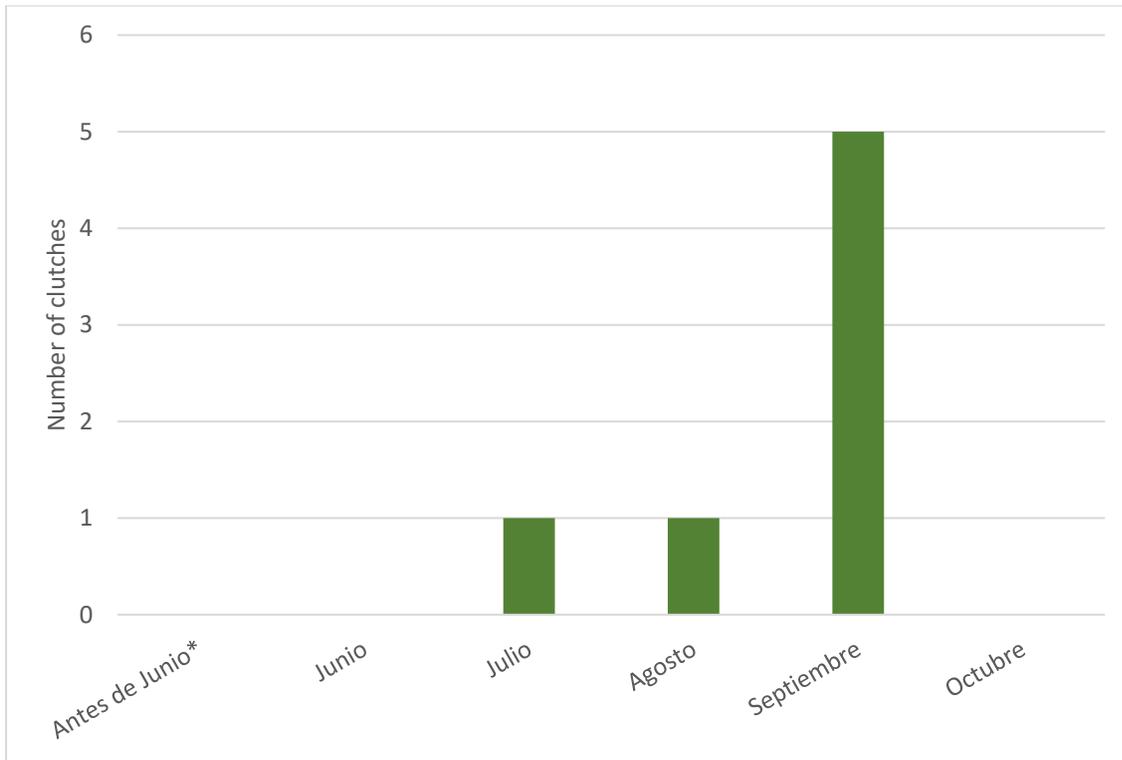


Figure 21. Nesting activity of the green turtle (*Chelonia mydas*) in the Gandoca-Manzanillo National Wildlife Refuge during the 2020 season. Number of Clutches per month. (* Data provided by the Gandoca Integral Development Association and the Gandoca sector park rangers)

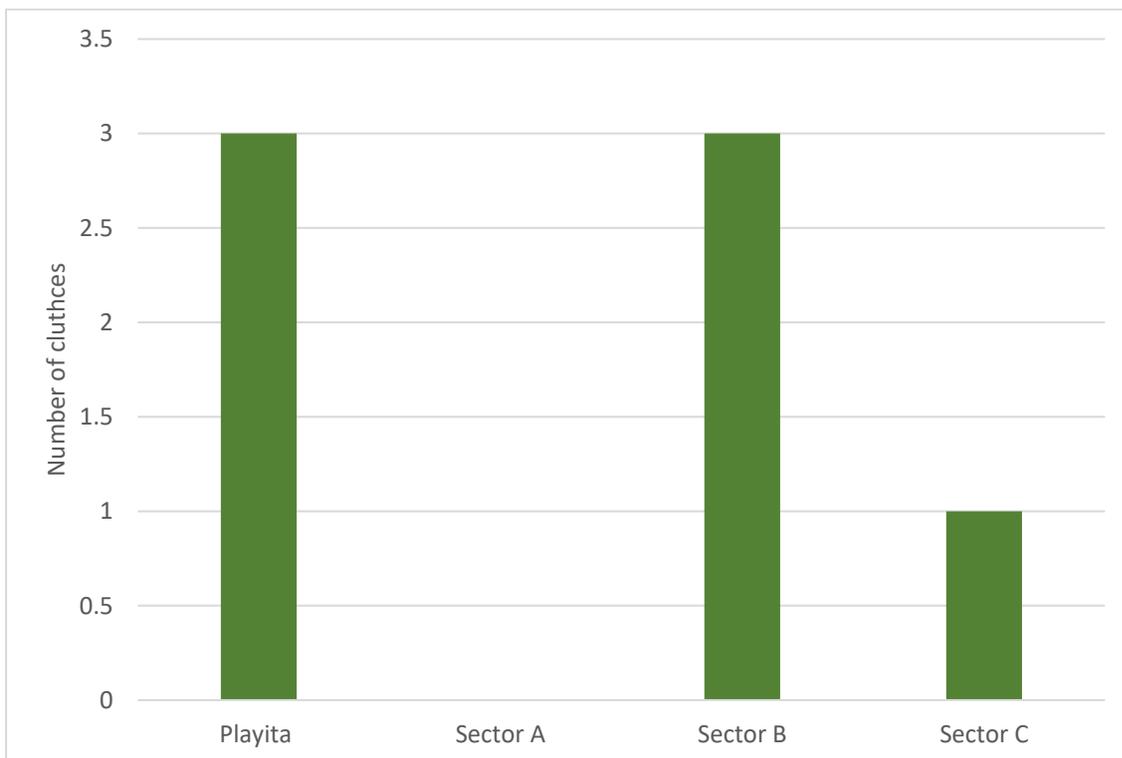


Figure 22. Nesting activity of the green turtle (*Chelonia mydas*) in the Gandoca-Manzanillo National Wildlife Refuge during the 2020 season. Number of clutches by sector on the beach.

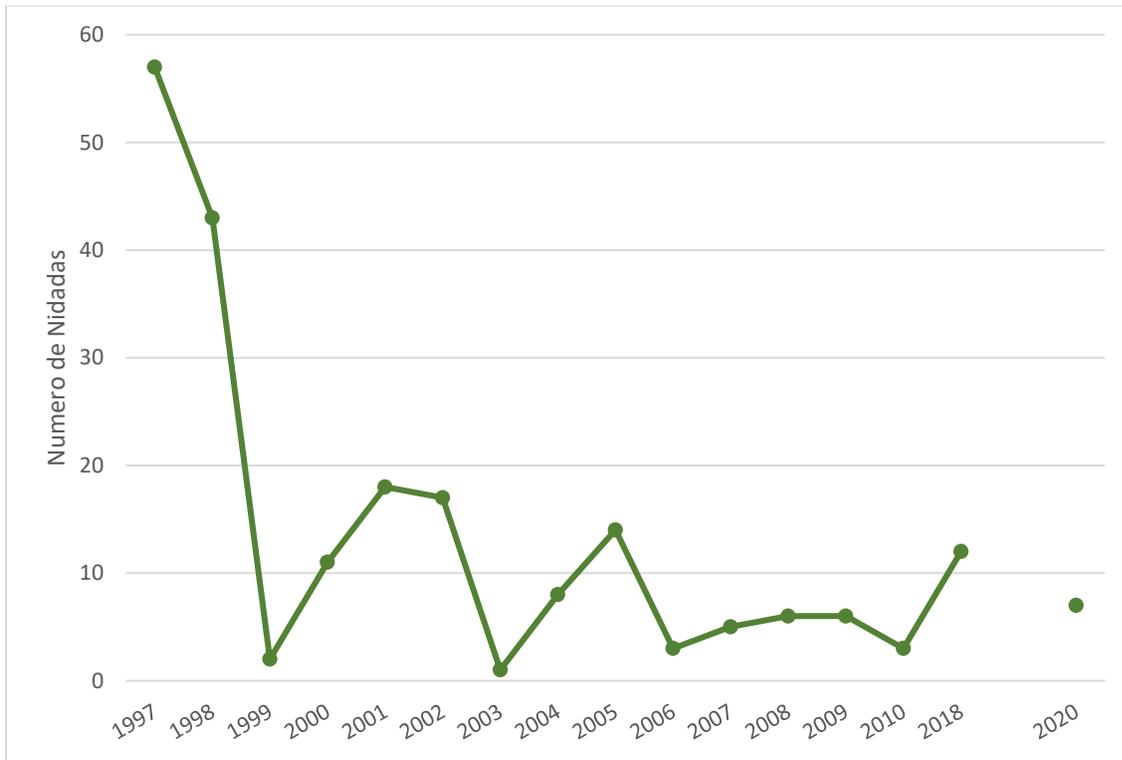


Figure 23. Nesting activity of green turtles in REGAMA from 1997-2020. Number of clutches per year. Large data gap from 2010 to 2018.

Clutch Fate

All clutches (n = 7) of the green turtle were successfully relocated to safer places on the beach, and none of these clutches were stolen by humans or predators (Figure 24).

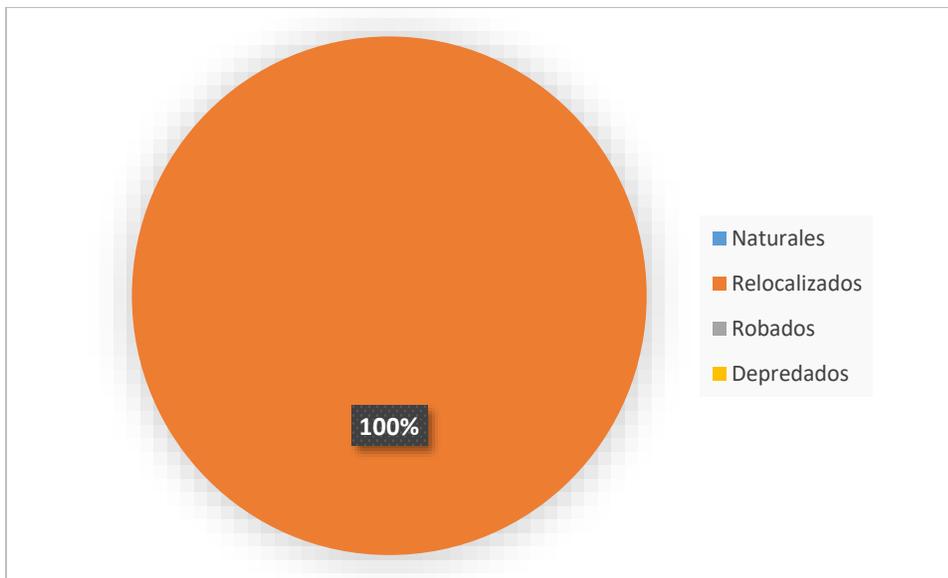


Figure 24. Fate of green turtle (*Chelonia mydas*) clutches during the 2020 nesting season in the Gandoca-Manzanillo Natural Wildlife Refuge. Naturales = natural; Relocalizados = Relocated; Robados = Poached; Depredados = Depredated;

Clutch Success and Number of Offspring Produced

The clutch success for green turtle clutches in 2020 was 100% (7 out of 7 nests hatched successfully).

The mean incubation period was 52.5 days (n = 4, range 48-57 days, SD ± 8.94).

We exhumed all green turtle clutches. The mean hatching success of green turtle clutches was 82.61% (n = 7, range 53.45-96.52%, SD ± 4.15). The mean emergence success was 73.27% (n = 7, range 9.48-96.52%, SD ± 33.04). In some nests, the hatchlings were trapped inside the nest and could not get out on their own because of roots inside the nest. Due to the exhumations that we carry out 24 hours to 48 hours after the majority of hatchlings has left the nest, many hatchlings were rescued and released.

The mean size of green hatchlings was 5.27 cm straight carapace length (SCL) (n = 1 clutch (10 hatchlings)) and 3.98 cm straight carapace width (SCW) (n = 1 clutch (10 hatchlings)). Green hatchlings weighed on average 27.83 g (n = 1 clutch (10 hatchlings)).

Of all the clutches laid, **603 green turtle hatchlings hatched and were released during 2020.**

Nesting Population Size, Marking-Recapture Results, Female Size, and Reproductive Output

Three different nesting females were identified during the season. All three females arrived without external tags and were tagged by us. We witnessed every single clutch being laid and we know that the seven nests were made by those three females that we identified and marked.

The re-nesting interval during the season was 12 days (n = 2, range 10-14).

The mean size of the nesting females was 101.28 cm curved carapace length (CCL) (n = 3, range 98.8-100.9 cm, SD ± 5.20) and 91.13 cm curved carapace width (CCW) (n = 3, range 86.6-97 cm, SD ± 5.44)

Female green turtles laid an average of 104.43 eggs per clutch (n = 7, range 75-116 eggs, SD ± 30.71) and **produced a total of 731 eggs during the 2020 season.**

Table 3. List of individual female green turtles identified in the Gandoca-Manzanillo National Wildlife Refuge during the 2020 season. Migratory status refers to three categories: females previously tagged in other seasons (remigrant - REM), females tagged in the same season but on another nesting beach (re-nesting - REN), and females tagged for the first time this season (recruit - REC).

Individual #	Left Tag	Right Tag	Migratory Status	Place Tagged (year)
1	0116CR	0119CR	REC	Gandoca (2020)
2	0033CR	0034CR	REC	Gandoca (2020)
3	0017CR	0018CR	REC	Gandoca (2020)

V.2. Foraging Activity

During the 2020 season, we realised a total of four boat trips to monitor the foraging activity of sea turtles in the REGAMA through transects in the reefs and seagrass meadows.

We were able to observe two juvenile hawksbill turtles and one adult green turtle during these surveys. In future, we would like to quantify the presence of turtles better and also try to capture turtle to record more data about the individual sea turtles and different life stages using REGAMA as foraging grounds.

VI. MANAGEMENT RECOMMENDATIONS:

Our report shows that REGAMA still harbours critical nesting habitat and very probable also foraging habitat for sea turtles. We recommend continuing to monitor these activities in the long term. For the year 2021, we also recommend that monitoring should start in March to include leatherback turtle nesting activity, and protective measures can be implemented, the same as for the hawksbill and green turtles in the year 2020.

For the future, we recommend mechanical measures to prevent predation of hawksbill clutches by raccoons and skunks.

In addition, to avoid the theft of nests by humans, the number of people working on the beach should be increased to cover the beach more often.

Also, the constant support of the park rangers in conjunction with reinforcement from the local police, coast guards, or similar would be invaluable for future seasons to prevent unauthorised people from entering the beach at night.

Cleaning the beach is an issue that needs more attention because there are no constant efforts to remove driftwood and plastic debris preventing the turtles from nesting and the newborns from reaching the water. In addition, plastic waste brought in by visitors and by the sea often remains on the beach if there is no more cleaning during the year. We recommend continued collaboration with the Gandoca community to clean the beaches frequently and find a way to improve the management of garbage that visitors leave on the beach.

Given all of these factors, we would like to apply for a renewal of our research permit for 2021.

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VIII. APPENDIX I: SOCIO-ECONOMIC IMPACT OF PROJECT

The non-profit organisation *Costa Rican Alliance for Sea Turtle Conservation & Science (COASTS)* and the social enterprise *Nāmaka Conservation Science*, through their activities and projects, have an impact on the scientific community, the conservation community, but also on the communities in which they work and carry out projects. We are promoting research and conservation of sea turtles and are functioning as a generator and transmitter of scientific knowledge in the field of marine biology. Moreover, we are also implementing concrete actions for the protection of sea turtles through community participation. Both COASTS and Nāmaka provide meeting places for marine conservation professionals and rural communities in Costa Rica, playing a crucial role in advising public bodies in charge of making decisions on the matter of sea turtle conservation. All of the above has one purpose, which is to conserve sea turtles for future generations while generating a positive social and economic impact for nearby communities.

We prepare this part of the report to make relevant and detailed information available to our stakeholders about our direct and indirect impacts on the Gandoca community during 2020, from a socio-economic point of view. Likewise, this report was also prepared and in order to ensure transparency in the management of funds granted to COASTS / Nāmaka.

The direct impacts of the project are mainly through the salaries that we pay to our local research assistants for *servicios profesionales*. Also, through paying room and board for our international assistants that either stay in local families or, as was the case in this season, in the project where we then hire a local cook.

We hired four local research assistants for five to seven months and paid a cook to cook for four international assistants that stayed in the project house for five months.

Salaries for local research assistants	US\$ 15,520
Salary for cook	US\$ 2,600
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TOTAL	US\$ 18,120

IX. APPENDIX II: HABITAT RESTORATION

We removed 168 kg of plastic debris during the 2020 nesting season.