Project Final Report

Research and Conservation Project of the Green Turtle (*Chelonia mydas*) and Hawksbill Turtle (*Eretmochelys imbricata*) in the South Pacific of Costa Rica

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Summary

Limited quantitative information is available for hawksbill turtles (*Eretmochelys imbricata*) at foraging grounds in the Eastern Tropical Pacific (ETP), where the species composes one of the most endangered marine turtle populations on the planet. Between August 2010 and March 2013 we captured individual hawksbill turtles using entanglement nets along the edges of mangroves and seagrasses of the Golfo Dulce, in southwest Pacific Costa Rica. A total of 62 hawksbills were captured, including 14 recaptures, of which 46 (74.19%) were juveniles (CCL <66 cm) and 16 (25.81%) were adults. The catch per unit effort (1 unit: 100 m of net for 7 hrs) during the study ranged between 0.03 and 0.07. The Golfo Dulce is highly turbid during the rainy season (May - November), particularly at our study area, as high sediment loads due to intensive runoff lead to poor water clarity. The probability of detection of hawksbills was considerably higher in the dry season (December - April) compared to the rainy season, suggesting these turtles may prefer waters with higher clarity. None of the individuals captured had evidence of internal or external tags, making it possible to conclude that they had not been previously marked at other feeding or breeding sites. A total of 28 (45.16%) individuals were found to host the ectoparasitic barnacle *Stephanolepas muricata*, which in high concentrations can be harmful by limiting the mobility of organs and limbs. Although consistent in-water quantification of hawksbills in the ETP remains scant, this study represents the longest and most robust marine monitoring dataset for hawksbills in the region to date. Our findings highlight the relevance of the Golfo Dulce as an important foraging ground for hawksbill turtles in the ETP and emphasize the need to monitor and protect this habitat to aid efforts to recover this critically endangered marine turtle population.

The Golfo Dulce is one of the few tropical fjords in the world, and has recently been declared a Marine Area Responsible Fisheries (AMPR), harboring mangrove ecosystems, coral reefs and seagrass beds are important feeding areas for green turtles (*Chelonia mydas*). In this study we estimate the catch per unit effort (CPUE) and population structure in a site in the western sector of the Golfo Dulce. Between August 2010 and March 2013, were captured a total of 253 green turtles (including 20 recaptures) using entanglement nets. The annual CPUE (CPUE, 1 unit: 100 m of net for 7 hr) during the study ranged between 0.19 and 0.45, decreasing gradually over the years, possibly prompted by mass mortality occurred in January 2013. There was no difference in CPUE between the dry and rainy seasons. About 78.17% were considered adult females, with an average length of curved carapace (LCC) of 79.6 ± 0.9 cm. There were no recaptures of tagged females in Golfo Dulce in any nesting beach Eastern Tropical Pacific, but there was an individual from the Galapagos Archipelago. Our results suggest that the Golfo Dulce is an important area for green turtles, where individuals congregate for adults and subadults would feed on seagrasses and the fleshy parts of the mangroves. The area also represents a major challenge for international conservation, possibly because we captured adult females from nesting beaches of Panama and Colombia, which requires multilateral agreements that promote the recovery of the East Pacific green turtle. We suggest the continuation of this conservation project to use sea turtles as umbrella species to protect ecosystems in the Golfo Dulce.

Introduction

Sea turtles have an important cultural, ecological and economic value. Indigenous communities of the region, as well as more recent colonialists have benefited from the meat, shell, skin and oil of these turtles. Archaeological studies testify to the evidence of more than 1,000 captures per years. The negative effect of these historical captures without any regulation are even exacerbated by the causes of death that originated with the mid-twentieth century, which include: the incidental entanglement in fishing nets, the fragmentation of nesting and feeding grounds due to coastal development and increased tourism, as well as the diversification of human activities in coastal areas and in the ocean. Latter has caused them to be regarded as an endangered species, being included in Appendix I of the CITES agreement (Convention on International Trade in Endangered Species of Wild Fauna and Flora).

Because of many different reasons, sea turtles have captivated human imagination since ancient times. As nutritional, economic and spiritual support, diverse social groups from around the world have been part of the cultural framework of many coastal regions (Molina, 1981; Frazier, 1999). For example, archaeological investigations have revealed remnants of associations between sea turtles and human settlements in both continental and insular regions dating from 1380 BC A.D. to 1715. It is thus clear that sea turtles were an important component of the diet and culture of many of these ancient social centers (Wing y Reitz, 1982; Versteeg y Effert, 1987).

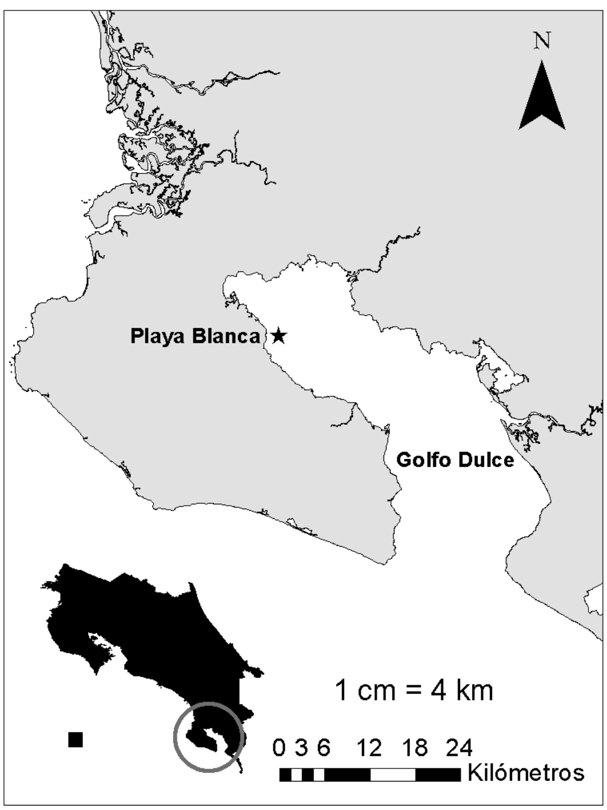

Figure 1: Map of Dulce Gulf, Osa region, Costa Rica.

In recent years, sea turtles have become the cause célèbre in many key issues of modern society, influencing the ways humans display and interact with their environment. Sea turtles play the role of a justice trial that illustrates the complexities associated to the development, continuation and promotion of biological conservation and environmental protection programs. These reptiles have been at the forefront – not by personal choice - on issues of great impact, as international discussions on trade and environment. For a better understanding of the connections between man and sea turtles, it is necessary, as a first step, to understand some basic features of these charismatic animals.

Hawksbill Turtle (*Eretmochelys imbricata*)

The hawksbill turtle is the only species of sea turtle that has a brightly colored keratinous shell, consisting of overlapping shields, known popularly as "shell". The keratin in the shell is also the main structural component found in nails and horns, which is why hawksbill shell is strong, even somewhat flexible. Hawksbill turtles are characterized by a pointed bill and narrow head, with what they can ideally reach their food resources often located in areas of difficult access. Furthermore, they have four lateral carapace shields, two pairs of prefrontal scales, and three post-orbital scales that distinguish them from other sea turtle species.

Figure 2: Captured hawksbill turtle in Dulce Gulf.

Hawksbill turtles can be found in tropical and subtropical waters of the world's oceans. It is thought, that after leaving their nests, hawksbills undertake a "frenetic swim" to reach oceanic areas in where they spend their early years to develop. After several years in the open ocean, they recruit neritic zones where they spend most of their lives as juveniles and adults. The juvenile hawksbills are typically more sedentary than adults of other species, but despite of the migrations of thousands of kilometers undertaken by adults, this species is considered to be typically less migratory than other sea turtles.

Hawksbills traditionally have been known to inhabit marine habitats of rocky substrates, whereas juveniles are more omnivorous, feeding on a great diversity of prey, while adults feed predominantly on sponges. In the Eastern Pacific, however, it has been documented that adults spend most of their time foraging in feeding grounds, that are located in mangrove estuaries (e.g. the Dulce Gulf), which could give a completely different perspective both for their habitat as for their diet. More research is needed to understand this aspect of their natural history in this particular region.

As all other sea turtles, hawksbills are threatened by the poaching of their eggs and meat for consumption and sale. This hazard is most pronounced in regions of extreme poverty, where turtles serve as an important source of protein and family income. Their bycatch in artisanal coastal fisheries as well as in industrial fisheries also means a big challenge, for one of which there is no easy solution as hundreds of thousands of people depend on fisheries for their livelihood. With the human population growing and an unstoppable and increasingly unsustainable coastal development, the destruction of key habitats for hawksbill nesting and foraging sites is similarly a huge issue. Other threats include pollution, a great scale change in oceanic nutrients and global warming.

However and exclusively for hawksbills, one of the main reasons for their population decline is the collection of shells (locally known as carapace shell or "bekko") for the manufacture of handicrafts like cockfighting spurs, combs, glasses and other objects. Individuals are also stuffed and sold to decorate the walls of markets, restaurants and homes. The result of this "value" added to the carapace causes, that if a hawksbill were found, it would probably be slaughtered instead of being returned to the sea. Although the collection of hawksbill shells and has been outlawed in many parts of the world, a strong black market still exists.

Until recent discoveries from end of 2007, it was assumed that hawksbills were essentially extinct in the Eastern Pacific. Nonetheless, it was now revealed that hawksbills in this region use remote nesting beaches, often located in between estuaries, where they land at the foot of mangroves to lay their eggs and it seems, that hawksbills even use these mangrove channels for foraging. These unique patterns in the natural history of Eastern Pacific hawksbills thus might be the reason, why it has gone almost unnoticed for the last decades, but it also makes the conservation of the species in the region particularly complicated.

The Black or Pacific Green Turtle (*Chelonia mydas agassizii*)

The black turtle or Pacific green turtle is characterized by a carapace length of 80 to 100 cm and can reach a weight of up to 100 Kgs, although in Nancite Beach a minimum nesting size of 76 cm in Curved Carapace Length (CCL) has been observed (Luis Fonseca, comm. pers.)

Figure 3: Black turtle, caught in Dulce Gulf.

Green turtles reach sexual maturity between 16 and 25 years and females nest every two or three years. A single turtle can nest up to five times per season, at an interval of 12-14 days. Nesting season for this species in the Pacific lasts from September till March.

The nest chamber depth is of approximately 50 cm. The average number of eggs laid is from 65 to 87 and average incubation period varies from 42 to 62 days.

On the Pacific coast of Costa Rica, important nesting beaches include Naranjo, Cabuyal, Carate, Punta Pargo, Nombre de Jesús and Río Oro, among others.

Black turtles are mainly herbivorous, feeding on algae and seagrass. Important foraging grounds comprise the proximity of the Galapagos Islands, as well as the seagrass beds and mangroves of the Dulce Gulf. Turtles foraging at the Galapagos Island undertake long journeys between to their nesting sides, whereas tagged turtles can be found in Mexico, El Salvador, Nicaragua, Costa Rica and Colombia.

Black turtles are also endangered, mainly due to the consumption of their meat and poaching of their eggs, as well as they are caught as bycatch. On some beaches of the Costa Rican Pacific, in recent years dozens of dead turtles have appeared, where in most cases human interaction was attributed, either because of observed abdominal injuries, or due to evidence of entanglement and hooks caused by fishing gears, together with some apparent poisoning during red tides.

Location:

Osa Sea Turtle Conservation Project: The *Osa In-Water* project is located on Playa Blanca, close to Puerto Jiménez on the Peninsula de Osa in the southern pacific province of Puntarenas, Costa Rica (Figure 3). Puerto Jiménez is on the Dulce Gulf, the bay between the Osa Peninsula and the mainland of Costa Rica. The surrounding landscape is flat farmland, but most of the Peninsula de Osa has been under the protection of the Corcovado National Park since 1975 when logging became a serious threat to the area. This national park is one of 25 biodiversity hotspots worldwide, with a vast variety of mammals, birds, amphibians, reptiles and insects. You may also spot dolphins and whales in the Dulce Gulf.

Figure 4: Map project location

Methods:

For the duration of one year, a total of 94 sets or nets were casted to catch turtles. All sets were performed by casting the 250 m long and 6 m high net in parallel direction to the coast. 10 different locations were tested, with the occurrence of the seagrass genus *Halodule* sp., *Halophyla* sp. and a combination of sandy and muddy sea bottoms.

Figure 5: turtle net in Set 1, Dulce Gulf.

In every set, the net was casted for seven hours, always beginning with a cycle of high tide. All sets were placed in the interior zone of the Dulce Gulf.

Once the net was casted, it was corrected by free diving, verifying that the entire line was well laid out from the water surface until the bottom. Subsequently, the boat with an average of seven researchers, which included the captain and assistant, withdrew itself to a distance of no more than 500 m and waited until a turtle was caught in the net.

In the manufacturing of the net, plumbs were used with a sufficient weight to prevent the current from lifting it up but at the same time were necessarily light to allow the turtles to reach the surface and to breathe while being entangled.

Once the turtle emerged and stirred up the water surface, the boat approached the net and the turtle was untangled and taken aboard. Once on the boat, a damp cloth was placed over the turtle's head, with the intention of covering its eyes and to stay calm. Afterwards, the turtle was positioned inside a mesh to immobilize it and sustained on a soft surface such as a rubber mattress or tire to not pressure its organs. In this position being positioned in the shadow and the dumb cloth on its eyes, it was proceeded to take its biometrics, blood samples, parasite samples and to apply an external tag, in case it didn't had one already, as well as a microchip. All recaptures were re-measured.

If the turtle showed high incidences of parasites (fig. 5), it was taken to a water tank where the turtle was submerged into a freshwater bath to kill the parasites by an osmotic process, after which they were extracted and the turtle sterilized with Vanodine 5%.

Figure 6: Parasites embedded deep in the skin and muscle of a hawksbill turtle

Upon completion of each procedure the turtles were returned to the sea. A data sheet with all information was filled out for each animal and digitally stored.

Sampling was concentrated basically on the inside of the Dulce Gulf and most samples were taken from site named Set 1 or around its vicinity, due to a cost-benefit matter as this site was on of the areas where most turtles were captured during the seven hours the net was set.

It is also important to considerate that the set locations coincided with those areas that where mostly frequented by turtles equipped with satellite tracking devices, allowing us to identify with certainty that we are sampling in the feeding ground of these organisms.

Results:

A total of 315 turtles of both species were captured, of which 253 were green turtles and 62 were hawksbills, with several 14 recaptures for Hawksbill and 20 for Green. The catch per unit of effort (CPUE) was 0.03-0.07 for Hawksbill and 0.19 to 0.45 for Green (number of turtles per 100 m net in 7 hours of set).

Hawksbill turtles represented around 20% of all captures, which shows its lower abundance in the study area.

Biometric records confirm that over 80% of captured green turtles had a carapace size greater than 76 cm, which is considered as the minimum nesting size for this species registered in the North Pacific of Costa Rica. Several specimens showed macroscopic evidence of sexual differentiation towards being a male, whereas 198 of the individuals captured were presumed to be immature males or females (fig. 8).

For green turtles, the curved carapace size class from 75.1 to 80 cm was with 45 records the most frequent, while the average length was 79.6 (SD = 0.9) with a maximum of 102, 2 cm and a minimum size of 47.6 cm.

In the case of hawksbill, the 74% were juveniles and 25.8% were adults. A total of 45.16% of organisms of *E. imbricata* showed *Stephanolepas muricata* associations, a variety of drill barnacle species that parasite massively and might even cause the turtles death (fig. 7).

Figure 7: Hawksbill turtle infected with *Stephanolepas muricata.*

Individuals that were highly infected and because of their conservation status of being critically endangered, were taken to tanks and for 24 hours immerged in a fresh water bath, in order to kill and extract these parasites carefully with pincers, with the open wounds being sterilized. Latter recaptures shows no re-infections at least for the next four months.

A relevant fact is that two green turtle specimens (LCC 88) were registered with tags originally from the Galapagos Islands, Ecuador. This information was confirmed with the Charles Darwin Foundation who reported that these females were registered in 2007 while nesting on Playa Quinta, Isla Isabela.

Blood samples for cell counts and chemistry analysis were taken from all turtles, whereas the data obtained in veterinary laboratories of the National University are not yet fully evaluated in, averages values are shown in table 2. During this year of study and conservation activities not climate extreme event happens to monitoring the effects of sediments and other threats over the feeding sea turtles habitats.

Two adult female green turtles were equipped with satellite transmitters with carapace sizes greater than the minimum nesting size reported for the country (76.0 cm). Their registered tracks are shown in figure 9.

No DNA or other genetic studies were done because of limited budget but we found support at the end of this project and we expect genetic studies report in 6 monts (June 2014). Nonetheless, ideas about investigations on hormones and stable isotopes of carbon and nitrogen for trophic analysis are explored.

During the entire study, there was no fish bycatch mortality. The only species captured not targeted were a total of 7 types sting rays that were released alive and didn't suffer any harm.

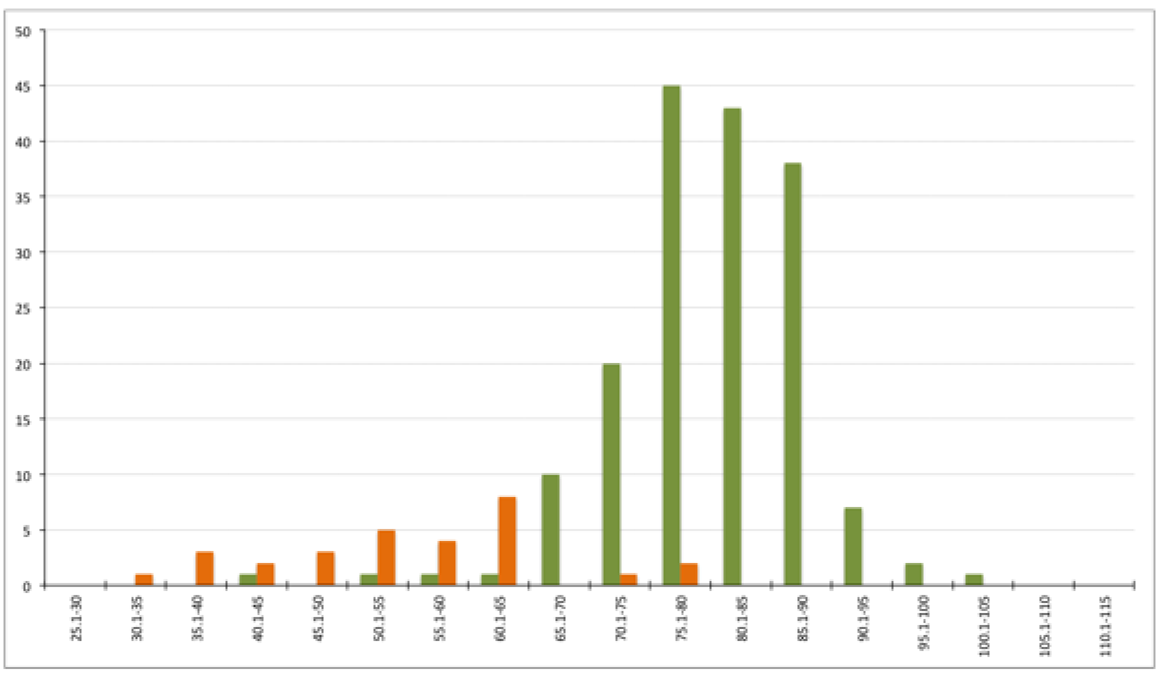

Figure 8: Distribution of turtles captured in a period of one year divided into size classes (Green: green turtles, orange: hawksbill turtles).

For now, values of chemical analysis and cell count of blood samples do not confirmation any alteration due to environmental stress or greater physiological changes. However, there are only few data available to comparison considering these topics.

One of the major benefits of this information is to be able to establish the basic parameters of wild populations, which then can be compared to the condition of individuals in captivity that are undergoing rehabilitation. In that way, our work contributes to science by establishing the baseline values in area of conservation biology.

Table 1: Chemistry and cell count in blood samples from turtles of the Dulce Gulf.

Blood chemistry (both species)	**Values (range)**
Total Protein	4.3 - 6.7 g/dl
Albumin	1.5 - 2.9g/dl
Globulin	1.2 - 4.1 g/dl
Glucose	58 - 127 mg/dl
Cholesterol	72 - 257 mg/dl
Urea nitrogen	1 - 16 g/dl
Creatinine	0.1 - 0.7 mg/dl
Phosphorus	6.6 - 13.4 mg/dl
Calcium	5.8 - 10.2 mg/dl
Magnesium	6 - 13.3 mg/dl
Uuric acid	0.7 - 2.1 mg/dl
Triglycerides	67 - 208 mg/dl
SAP	91 - 298 U/L
ALT	1 - 13 U/L
Cell count	**Value range**
Hematocrit	35 - 42%
Hemoglobin	10.8 - 14.4 g/dl
MCHC	32 - 36
Leukocyte Count	889 - 2560 ul
Heterophile	861 - 1091 ul
Eosinophils	44 - 231 ul
Basophils	0 - 15 ul
Lymphocytes	55 - 451 ul
Monocytes	11 - 64 ul

The two females of adult carapace size, which were equipped with satellite transmitters (September 2010 and November 2011), showed what we have called a feeding behavior and a habitat use for this purpose. Several important data derive from this information associated to the integrated management of the Gulf and its food web, as the data show that these individuals even travel upriver and incorporate especially mangrove areas in their foraging movement pattern, which ultimately leads to determine the importance of these ecosystems and the need to extend conservation efforts with the aim to maintain the ecosystem structure of these coastal forests.

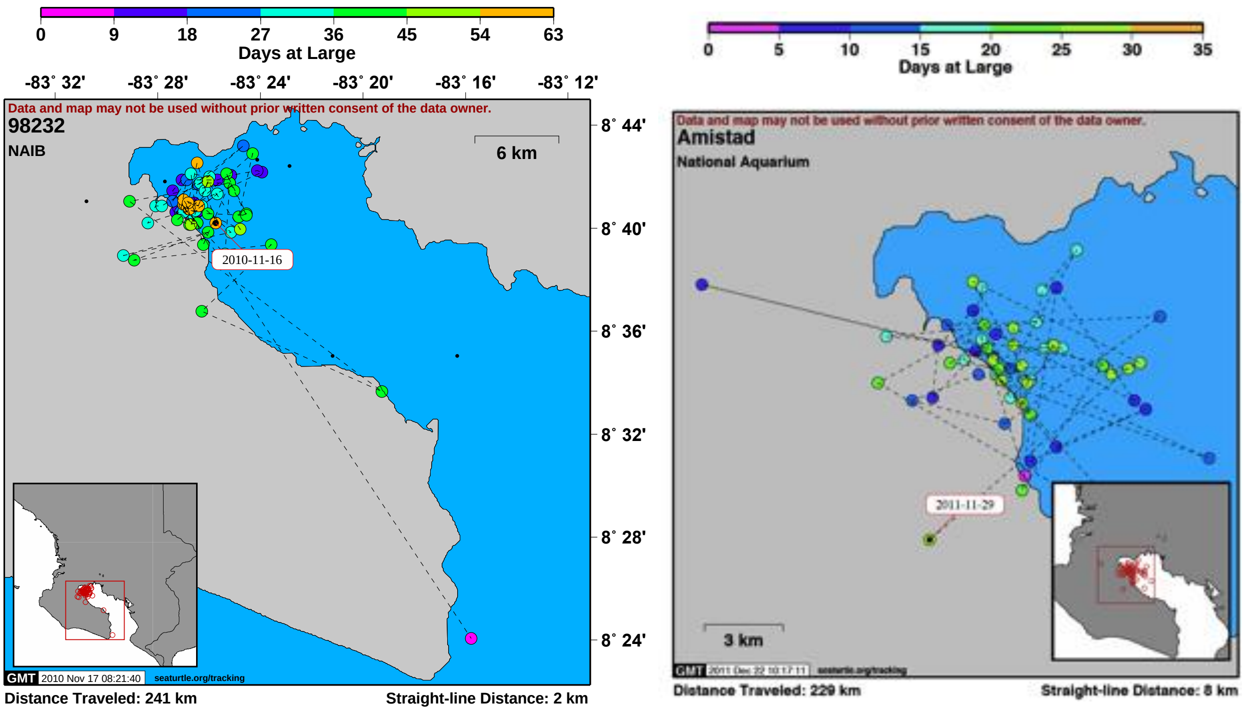

Figure 9: Migratory movements of two green turtles in the foraging area studied within the Dulce Gulf tagged in 2010 and 2011. The almost identical movements of different animals are evident.

The obtained results not only allowed gaining more information on the health characterization of green and hawksbill turtle populations, but also to describe health problems and to propose procedures for possible solutions. These data also showed the importance of the marine areas of the Gulf in connection to the feeding grounds for nesting populations such as from the oceanic islands like the Galapagos Islands. It is worthwhile to continue this investigation, tagging these animals to obtain recapture data from nesting beaches and in that sense to close the lifecycle of these species and to identify the location of their critical habitats.

The methodology applied proves to be completely friendly with other species, since no mortality in other species including fish, dolphins, etc. was registered.

Capture rates show a greater abundance of turtles of the *Chelonia* genus compared to *Eretmochelys*, also that the Green turtle population if main represented by adult specimen and for hawksbills the population is represented mostly by juvenile specimens, shown different use of the marine ecosystem by each specie.

During this year the project also operated a mangrove plant nursery (Fig. 10) to help this critical habitat to recover, over 10000 plants were growth up and were planted in around 2 km of coast front line in the internal area of the Dulce Gulf. For this work WIDECAST recruited around 200 volunteers (international and national) to take care the plants and to make the reforestation on muddy areas.

The WIDECAST Team, also design and test the methodology to record the condition and biomass of the sea grass in the area (Fig. 10b). This work starts with the participation of a student from San Diego, California and now we monitored 6 transepts with at least 5 pins that include 4 quadrats each, soon we will have more information about condition and biomass of this important ecosystem.

Figure 10: Mangrove nursery in Osa Project

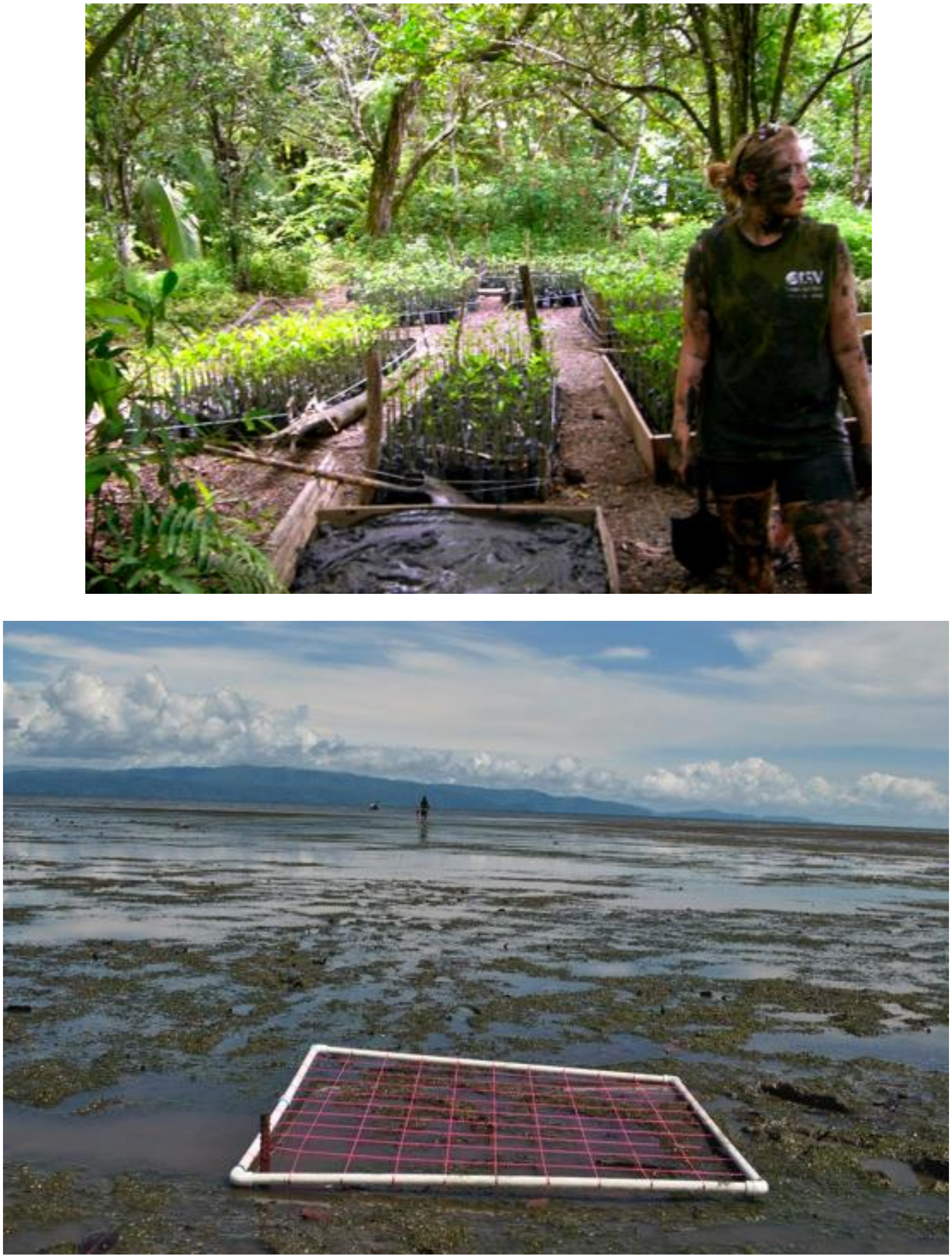

Figure 11. A. Filling the plant boxes with marine mood. B. Installing pins and quadrats on the sea grass during the full moon low tide for further studies of this important feeding critical habitat.

The activities of the project focus on Sea Turtles and the critical habitats also supported work with some threats as plastics in the stomach, wounds, intestinal obstructions, boat crash and float problems as the most common injuries in the rescue turtles. In total we rescue and release 35 sea turtles during 2012 and 20 during 2013, this amount do not include the hawksbills with parasites.

The team project also visit the La Palma and Playa Blanca primary schools and work with the kids teaching them about the turtles and the importance of the conservation of the marine critical habitats. An estimation of 150 children participate in our environmental activities, each receive an activities manual, 2 presentations and 2 hands on activities like sea turtle release or rescue center visit.

To increase our reach and positive impact local and globally we based some of our activities in the Voluntourism, ecotourism to increase the visit to Playa Blanca location and increase the income in the home stay families and volunteers to increase the frequency and the reach of our conservation activities.


Figure 12: Volunteer in front of our rescue center, Veterinary students learning about sea turtles and local school kids receiving our project presentation.

Here are remarkable outcomes:

- 2011, we start the in water monitoring under the Fisheries Office, permit Number AJDIP-127-2011. In other words, is an activity with all permits. Also we have permits from MINAET to sampling in protected marine areas.
- 2012, January we with the help of several people, sponsors as PTES and volunteer fees build up the facilities. Now we rescue and rehabilitated greens, olive ridleys and hawksbill turtles
- This project have 3 focus areas: 1. In water monitoring, 2. Habitat rehabilitation (Mangrove and sea grass), 3. Rescue Center.
- Our General Goal is: Improve the conservation status of the sea turtle in the first responsible fishing area of Costa Rica.
  - One master student doing thesis with tissue samples for DNA studies (Alberto Abreu Lab.) and Isotopes N-C (Jeff Seminoff Lab.)- Maike Hidermayer (under execution)
  - One student from national vet school developing hormones study for her thesis with the blood samples.-Priscilla Howell- (done)
  - Mapping the sea grass areas as feeding zones in the internal region of Dulce Gulf. Student: Ricardo Espino. (done)
  - Blood analysis for 150 green and 30 hawksbills, we are looking for any differences in their health before and after the climatic stress of their critical habitats (Sea grass and mangroves)
  - 315 green turtles and 47 hawksbill sampled, tagged (NOAA inconel tags and our PIT tags), and studied. 25 of hawksbill were putted in fresh water bath in average 2 days to remove the ***Stephanolepas*** which are killing them.
  - Two green turtles tagged with satellite tracker.
  - Two scientific articles submitted and approved in the Tropical Biology Journal.
  - Two turtles trapped with Galapagos Island tags, they were nesting in Isabella Island in 2007.
  - CPUE, abundance a population description was developed and compared

with others in the Tropical Eastern Pacific.

- More of 10000 mangrove trees planted and one plant hatchery in operation.
- More of 50 km of beach clean up in support of the Blue Flag local committee.
- More of 550 volunteers living in at least 6 local facilities producing more the $40,000 as alternative livelihood. (2010-2013).
- Project gave job for at least 10 people between boat captain, boat helper, local supplier, carpenter, etc.
- One agreement with NIPRO Company to receive equipment. Conservation International is supporting the local alternative livelihood on voluntarism project.

Next step:

For a continuation of the project, a bi-weekly monitoring of respectively seven hours of net deploy is proposed in the inner zone of the Gulf until gaining a recapture rates of 95% that has still not been achieved for either species. This sampling model will be adaptive to the personnel available as well as to the weather and especially to the condition of the sea.

More frequent sampling could be realized depending on the resources available. Each survey is completed by using the vessel called **WIDECAST**, being 8.15 m long, 1.80 m wide and 0.72 m high, with an engine brand MERCURY series 1B857566 and registration number PG-9267 and its departure port being Playa Blanca de Osa at 3 km from La Palma.

Our plan is not only be the unique option for sea turtles with health problems in Costa Rica also we want:

- Be a training center for marine biologist and veterinaries (in operation)
- Develop studies on the critical habitats as Sea Grass, Mangroves and Pacific Coral Reef.
- Study the choice to develop a pilot project with "Bio-Rock" to recovery the reef around Playa Blanca.
- Install satellite tracker in males for both species.
- Develop a health data center for both species to be used by local and international partners including DNA, blood, stomach contents and other.
- Increase the medical procedures precision to manipulate sea turtle health problems and share with the national and international society.
- Create a rescue and rehabilitation network in Costa Rica Pacific coast.

- Purchase a new outboard motor and boat.
- Purchase a piece of land to build up our facilities in Playa Blanca

We are developing an effort to give stable and permanent resources to the project by support from proposals, volunteer fees and sea turtle adoptions.

Finally, we appreciate all support receive by PTES to this project, particularly their special consideration to help our project in this area by continuation funds.

This is Piña our last rescue in 2013, now she is healthy and ready to be rehabilitated to released.