TECHNICAL REPORT

SCIENTIFIC RESEARCH PROJECT AUTHORIZED BY THE GALAPAGOS NATIONAL PARK DIRECTORATE

1. RESEARCH TEAM

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2. PROJECT DETAILS

Research Permit **PC-53-19**: Sharks of the Galapagos Marine Reserve (GMR): Assessment of population status and habitat use, connectivity and migration routes.

Technical report of the hammerhead shark diet (*Sphyrna lewini*) research project throughout its ontogeny in the GMR.

3. SUMMARY

This report details the main activities carried out in project PC-53-19 between the period of January-October 2019 in the line of research on the diet of hammerhead sharks (*Sphyrna lewini*) throughout its ontogeny through the use of stable isotopes. The trips and main activities are detailed in Table 1. Currently, the analysis of the information collected is being carried out since many of the components are long-term studies or with insufficient information to present final results.

ACTIVITIES AND TIMESCALE

Table 1. Fieldwork carried out during the months of January-July 2019.

Days	Sites	Activity
31/01/19- 10/02/19	Darwin and Wolf islands	Obtained shark biopsies from adult and sub-adult hammerheads.
07/06/19 – 14/06/19	Venecia and Caleta tortuga Negra	Sampling around the nurseries to obtained biopsies of neonatal and juvenile hammerhead sharks
17/06/19 – 29/06/19	Galapagos Science Center - USFQ	Laboratory analysis of hammerhead shark tissue samples

1. BACKGROUND INFORMATION

The Galapagos Islands are home to large numbers of predators, such as the hammerhead shark, *Sphyrna lewini*. This species is classified as "Endangered" by the IUCN Red List. Hammerhead sharks are migratory and have high connectivity between Galapagos in Ecuador and other oceanic islands within the Tropical Eastern Pacific (Bessudo et al. 2011). However, despite the protection of the Galapagos Marine Reserve (GMR), hammerhead sharks remain vulnerable to legal and illegal commercial fishing when they travel outside these limits. In addition, sharks are also affected by the climatic variability that occurs in the Tropical East Pacific during El Niño. Some of the impacts include rising sea surface temperature, stratification of the water column and increasing incidence of diseases, and these are related to alterations in hammerhead shark populations (Klimley and Jorgensen 2003) . Several studies have predicted that climate change will increase the frequency and intensity of El Niño phenomena (Cai et al. 2014). According to NOAA, there was a strong El Niño phenomenon in the years 2016-2017 (Figure 1) and another event is predicted for 2019. El Niño significantly affects the structure of pelagic ecosystems, limiting the habitat available to predators and changing the biomass of fodder groups, including small fish and cephalopods, which are an important component of the hammerhead shark diet (Torres-Rojas et al. 2006).

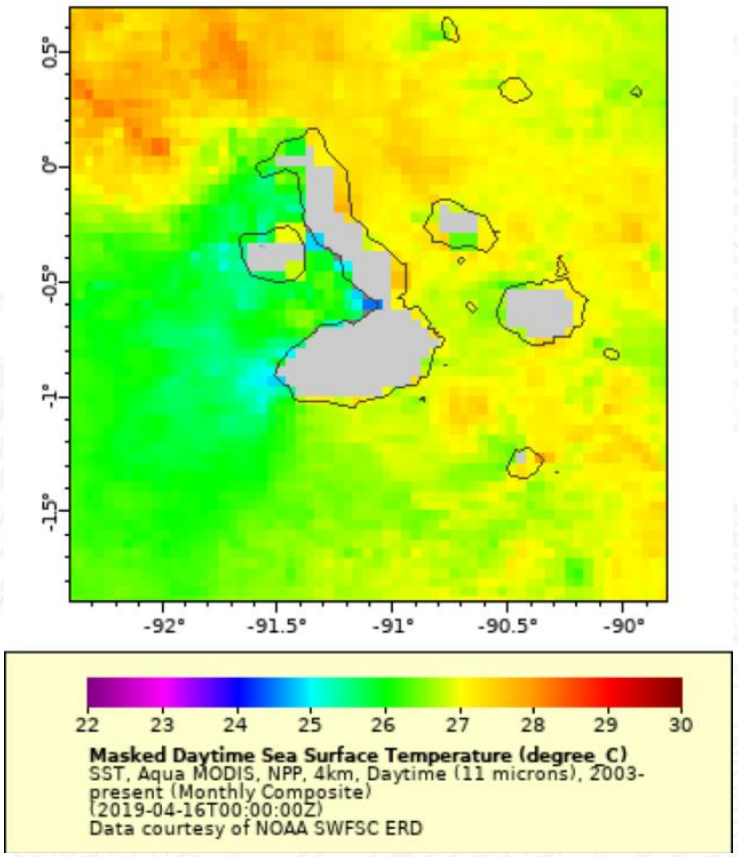

Figure 1. Map of the Galapagos archipelago showing the average sea surface temperature in degrees Celsius from 2003 to 2019. Source: NOAA.

2. GOALS AND OBJECTIVES

To study the impact of climate variability on the feeding behavior of the hammerhead shark by analyzing stable isotopes in tissue biopsy samples obtained during variable climatic conditions (El Niño 2019 phenomenon) and perform a comparative analysis with previous samples obtained during years of normal climatic conditions (2016 and 2018).

SPECIFIC OBJECTIVES

(1) Obtain at least 30 tissue biopsies of juvenile and adult hammerhead sharks during the planned El Niño event of 2019 at the Galapagos Marine Reserve.

(2) Perform stable isotope analysis on the samples collected in 2019 and the samples previously collected by the Galapagos National Park rangers and other researchers between 2016 - 2018 (unpublished data)

(3) By analyzing stable isotopes determine the feeding sites and trophic role of hammerhead sharks within the RMG

3. METHODS

Obtaining hammerhead shark biopsies

We obtained the hammerhead sharks biopsies through the use of Hawaiian rods equipped with a specially designed biopsy dart (Pneu-Dart, USA). While a free diver descends to a depth of 5-15 m to take the sample from the shark's dorsal fin, his/her partner is floating on the surface for safety reasons, observing and supporting the diver who descends. The samples are mainly taken from the caudal or dorsal fin, since it is the part of the shark's body where the tissue is most effective for analysis. The tissue samples are cut in two and preserved in sterile Eppendorf's vials in a freezer within 3 hours of obtaining them. Each sample is preserved in 70% ethanol for stable isotope analysis.

Stable isotope analysis methods

Obtained tissue samples are rinsed with deionized water to remove debris that could alter the isotopic signature. The samples are then placed in glass vials previously treated for 24 h with a mixture of chromic acid prepared from sulfuric acid and potassium dichromate. The samples are then dried in a desiccator at 80 ° C for 12 h to remove all moisture. A microwave-assisted extraction protocol (MAE) (microwave oven model: MARS 5x 1000 W, CEM, Matthews, USA) is applied using 25 ml of a 1: 1 chloroform / methanol solution and dried again (Tieszen et al. 1983). The samples are homogenized with an agate mortar to obtain a very fine powder, of which ~1 mg will be weighed by means of an analytical micro balance with an accuracy of 0.001 mg and transferred to a capsule of Tin for isotopic analysis. The stable isotope ratios δ13C and δ15N are determined by a mass spectrometer with PDZ Europe 20–20 continuous flow isotope ratio (Sercon Ltd., Cheshire, UK) in the stable isotope facility of the University of California Davis (California, USA). Finally, the results, expressed in parts per thousand (‰), will be obtained using the following equation: δ13C or δ15N = 1000 ([Rsample⁄

Rstandard] - 1), where Rsample and Rstandard are 13C / 12C or 15N / 14N Sample and standard proportions, respectively. The standards are Pee Dee Belemnite (PDB) for δ13C and atmospheric N2 for δ15N.

4. RESULTS

PRELIMINARY RESULTS

Obtaining hammerhead shark tissue samples in Darwin and Wolf islands

We sampled on the Darwin Island's arch platform during a fieldtrip in February 2019. A total of 16 samples were obtained, mostly adult females > 2.2 m long (Table 2). For subsequent analysis of stable isotopes, previous samples of adult hammerhead sharks that were also taken in January 2018 at the same site were included in this analyses (Table 2).

Table 2. Adult hammerhead sharks (*S. lewini*), sampled in the months of January 2018 and February 2019.

Site	Sex	TL (m)	Date
Darwin and Wolf	Female	> 2 m	28/01/2018
Darwin and Wolf	Female	2.3 m	28/01/2018
Darwin and Wolf	Female	2.3 m	28/01/2018
Darwin and Wolf	Female	> 2 m	28/01/2018
Darwin and Wolf	Female	> 2 m	28/01/2018
Darwin and Wolf	Female	> 2 m	28/01/2018
Darwin and Wolf	Female	> 2 m	06/02/2019
Darwin and Wolf	Female	2.3 m	06/02/2019
Darwin and Wolf	Female	2.3 m	06/02/2019
Darwin and Wolf	Female	> 2 m	06/02/2019
Darwin and Wolf	Female	> 2 m	07/02/2019
Darwin and Wolf	Male	1.5 m	07/02/2019
Darwin and Wolf	Female	2.3 m	07/02/2019
Darwin and Wolf	Female	2.3 m	07/02/2019
Darwin and Wolf	Female	> 2 m	08/02/2019
Darwin and Wolf	Female	> 2 m	08/02/2019
Darwin and Wolf	Female	1.5 m	08/02/2019
Darwin and Wolf	Female	> 2 m	08/02/2019
Darwin and Wolf	Female	> 2 m	08/02/2019
Darwin and Wolf	Female	> 2 m	08/02/2019
Darwin and Wolf	Female	> 2 m	08/02/2019
Darwin and Wolf	Female	2.3 m	08/02/2019

Obtaining neonates and juveniles hammerhead shark tissue samples at the nurseries Field trips were taken to the bays of Santa Cruz Island, Venice and Caleta Tortuga Negra, in the month of June 2019 along with DPNG park rangers. The GNPD showed in 2017 that these sites are possible hammerhead shark nurseries. Based on these findings, and with the collaboration of artisanal fisherman Nelson Ybarra and GNPD park rangers, we place nets in strategic places within these bays to capture sharks and obtain tissue samples. A total of 8 samples of neonatal and juvenile hammerhead shark were obtained (Table 3), and these were mostly males. For subsequent analysis of stable isotopes, samples of neonatal and juvenile hammerheads were also taken in the months of May, June, August and November of the year 2018 in the same sites.

Table 3. Neonates and juveniles' hammerhead shark (*S. lewini*) sampled in the months of May, June, August and November 2018 and June 2019.

Site	Sex	TL (cm)	Date
Venecia	Female	70	25/05/18
Venecia	Male	68	25/05/18
Venecia	Female	73	25/05/18
Venecia	Male	64	25/05/18
Venecia	Male	60	25/05/18
Caleta Tortuga Negra	Female	58	18/06/18
Caleta Tortuga Negra	Male	63	18/06/18
Venecia	Female	72	11/08/18
Venecia	Female	70	14/11/18
Venecia	Male	78	14/11/18
Venecia	Male	68	14/11/18
Venecia	Male	82	14/11/18
Venecia	Female	63	14/11/18
Venecia	Male	63	07/06/19
Venecia	Male	65	07/06/19
Venecia	Male	75	07/06/19
Venecia	Male	89	07/06/19
Venecia	Male	61	07/06/19
Venecia	Male	64	07/06/19
Venecia	Male	44	12/06/19
Caleta Tortuga Negra	Male	85	15/06/19

Stable isotope analysis of hammerhead shark biopsies

A total of 48 hammerhead shark (neonates, juveniles and adults) tissue samples were taken to the laboratories of the Galapagos Science Center, where our USFQ collaborator Dr. Diego Páez-Rosas is based. In these facilities the samples were prepared for subsequent analysis of stable isotopes. The samples will be processed in the laboratories of the University of California Davis, possibly in the first quarter of 2020. The results of these analyzes will be complemented with results of stable isotopes from tissues collected during the years 2016 - 2017 (Figure 2a and b).

a)

b)

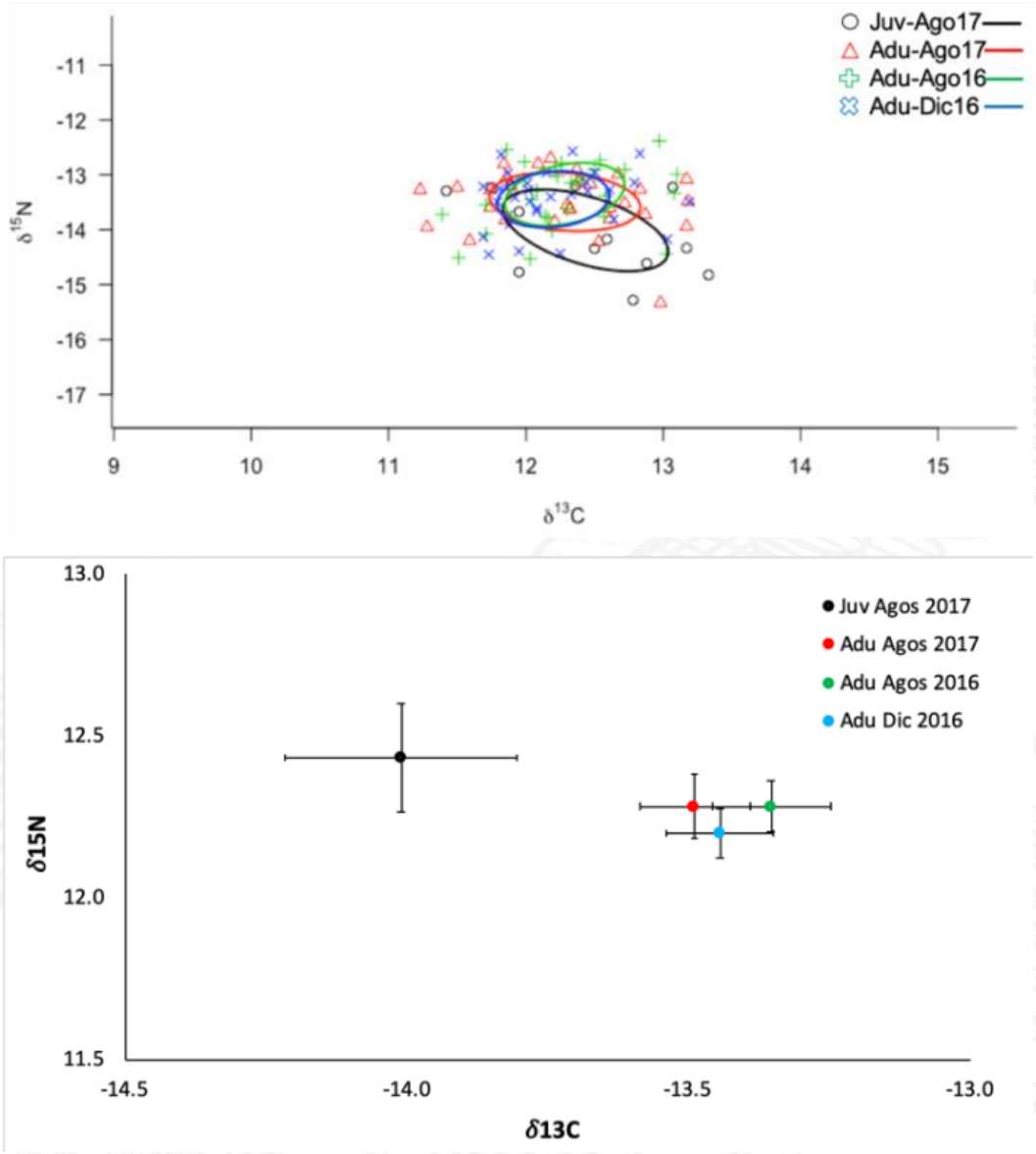

Figure 2. Differences in the isotopic footprint (C, N) of hammerhead sharks (adults and juveniles), from 2016 – 2017, obtained at Darwin and Wolf Islands.

Feeding behavior analyses results of hammerhead sharks during the years 2016 - 2017

During the years of this study a evident climatic variability has been observed (Figure 1) and the results of the stable isotope analysis on hammerhead sharks from the years 2016 - 2017 demonstrate that there are clear ontogenic changes in which adults do not alter their feeding strategies over time, while juvenile hammerhead sharks do alter their feeding behaviour, moving into pelagic waters to find food. Adults, however, spend most of their time feeding in oceanic less productive environments.

5. DISCUSSION

Currently, we have the results of the isotopic analyzes of 2016 and 2017 of adults and juveniles of *S. lewini* at the Darwin and Wolf Islands. However, at the beginning of next year 2020 we expect to have the results of the stable isotope analysis of the years 2017 - 2019 (100 additional samples) of neonates, juveniles, and adults sampled in the bays of Santa Cruz (Venice and Caleta Tortuga Negra) and Darwin and Wolf islands. Following the methodology previously used in by Paéz-Rosas et al. (2018), we will present the results obtained in a scientific publication that will be sent to an indexed journal. The preliminary results and methodology of this project were presented at the 60-year Science Symposium of the Charles Darwin Foundation and the Galapagos National Park as a poster in July 2019.

This project that aims to study the feeding behavior of hammerhead sharks of the GMR, seeks to form a baseline of 4 year period with data from different seasons (cold / hot) and different ontogenetic stages (neonate, juvenile, adult), to provide a clear vision of the feeding behavior of hammerhead sharks in the GMR. This is a pioneering study for the Galapagos Islands and it is suggested to continue with this methodology to create a more robust database and thus be able to make more informed decisions to ensure the conservation of this iconic species of the Eastern Tropical Pacific.

Previous studies have shown that hammerhead sharks are highly migratory and in order to protect their populations it is necessary to study the areas of greatest ecological importance for these species to take sound protective measures. In addition, hammerhead sharks are located in the upper levels of food chains and help maintain healthy ecosystems, therefore, their reduction has negative effects from top to bottom (Baum and Worm 2009) that cascade throughout the food web (Torres-rojas and Galvan-maga 2004). These sharks act as “umbrella” species, which means that studying and conserving their populations will also help protect other species located in the lower levels of the food chain. Finally, due to the high connectivity of the population throughout the Eastern Tropical Pacific, our findings may be related to other hammerhead shark populations, as these are equally exposed to the impacts of climate variability.

6. RECOMENDATIONS

With the climatic variations that occurred during the first semester of the year 2019 and the future predictions of extreme and frequent climatic variations, it is recommended to continue obtaining tissue samples and evaluating the feeding behavior

of the hammerhead sharks, *S. lewini*, within the GMR. It is also recommended to evaluate the areas of greatest feeding use of juvenile hammerhead sharks, since results from the years 2016 - 2017 demonstrate that they use deep pelagic areas for feeding. Therefore, it is recommended to evaluate and consider better protection measures for these areas.

7. ACKNOWLEDGMENTS

This project is a joint collaboration between the Charles Darwin Foundation, the Galapagos National Park Directorate, and the Galapagos Science Center of the San Francisco University. This project is supported by the Rufford Foundation. We like to give a special thanks to the fisherman Nelson Ybarra, and to the crew members of the Queen Mabel, as well as to all the volunteers, collaborators and park rangers of the GNPD who participated in the field trips; they have been an essential part of the team.

8. REFERENCES

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