Final Evaluation Report

Your Details	
Full Name	Yasuní Chiriboga
Project Title	Evaluating by-catch stress levels related to growth and survivorship of juvenile blacktip sharks in San Cristobal, Galapagos Marine Reserve.
Application ID	*29452-2*
Grant Amount	£6,000
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Date of this Report	27th January 2022

1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Characterize the physiological response in juvenile blacktip sharks following a gillnet capture, including environmental variables				95 sharks were tested for glucose, lactate, and haemoglobin parameters after entanglement. Fight time in the net was recorded, as well sea surface temperature (SST).
Characterize reflexes and swimming behaviour after a gillnet capture				From 95 assessed individuals, we had one dead shark post -release.
Evaluate juveniles' abundance by integrating benthic-BRUVS and drone technology				This new objective was proposed instead of the capture-mark-recapture objective.
Understand how fishing practices (fishing arts and fishing seasons) could be improved to avoid by-catch of blacktip sharks at early stages.				We will be holding talks with the Galapagos National Park Directorate to show the results of the study and suggest improved fishing practices for the site.
Involve and train local students to be an active part at the field and in the data analysis process.				We trained and worked with a local masters' student, as well as with local pre-graduate students.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.

Because of the COVID-19 pandemic we needed to reschedule activities and re-establish some objectives because of intense regulations regarding the number of people permitted on board and available sites to visit. Here are two important difficulties we faced, and the way we tackled them:

1. We were not able to carry out the capture-mark-recapture (CMR) study. The objective was to obtain information regarding growth, survivorship, and abundance during the season. Given that we sampled during the pick abundance of blacktips, this activity required a team of at least eight people on board (which was not permitted) to avoid mortalities of juvenile sharks.

We tackled this difficulty by replacing this methodology for benthic BRUVs and drone technology, which let us assess abundance and spot the specific sites at the bay where sharks were present (in a bay where fishing and tourism are the main

human activities). We note that this remains a priority and we will be carrying out a first mark-recapture assessment using counterpart funds in April 2022.

2. At first, two sites, Puerto Grande and La Seca, were the sampling objectives. During 2016 (1st Rufford Small Grant), we identified these two bays as the most important potential nursery grounds for blacktips around San Cristobal.
Nevertheless, we faced two difficulties regarding the sampling sites for this study:

Before the pandemic started, we were able to visit both places. While undertaking the short-term mortality component at La Seca (tracking individuals for 10 minutes to record the swimming behaviour post-release) we faced a substratum difficulty. The rocky substratum made it difficult to release the small visual float that was attached to the first dorsal fin because juveniles swam in between the rocks to feel protected. Even though we released all floats from sampled individuals, we knew that this would be a difficulty for future sampling.

By the time pandemic started, La Seca was one of the restricted places to visit around the island. Given this, we decided to intensify sampling efforts at Puerto Grande, place in which juvenile sharks (including the critically endangered scalloped hammerhead *Sphyrna lewini*) interact with tourism and fishing activities. Puerto Grande has a rocky-sandy bottom, having no problems while tracking the individuals and then releasing the floats.

3. Briefly describe the three most important outcomes of your project.

Coastal nursery grounds offer pups protection from predators, as well as food resources for their development. Despite this, young sharks are exposed to increased risk of by-catch by coastal fishing interactions. The physiological response that juvenile sharks exhibit to these stressful events (fight time in nets) includes a release of hormones (e.g., adrenaline), and the bioaccumulation of anaerobic metabolism products (e.g., lactate) resulting in ionic and osmotic imbalances (Bouyoucos et al. 2018). In addition to anthropogenic stressors, young sharks must deal with stressful environmental variables (e.g., variations in water temperature and salinity).
During this study, we found that:

1. An increased fight time in the net results in increased values of glucose and lactate concentrations. This is explained because vigorous attempts by sharks to escape from the net, as well as the restriction of oxygen to ventilate the gills, make sharks to rely on anaerobic metabolic pathways (Dapp et al. 2016; Bouyoucos et al. 2017). This increases blood glucose and lactate concentrations, declining blood pH and creating a scenario of metabolic stress. Additionally, we found that there are increased lactate concentrations in smaller individuals (<65 cm). This suggests that smaller individuals require higher energetic expenditure during fight time in the gillnet (Moyes et al. 2006) having a greater probability of mortality compared with larger young sharks.

2. Water temperature ranged in between 25 °C (February, warm season) to 22 °C (June, cool season). There was a significant and negative correlation in between water temperature and glucose concentrations ($p = 0.04 < 0.05$; corr = -0.21). This suggests that an increase in water temperature results in lower values of glucose

concentrations. Additionally, we found a significant difference when relating the lactate concentrations with water temperature and fight time in the net. An increase in water temperature and fight time in the gillnet, result in higher concentrations of lactate. This could be explained because some physiological parameters could change when water temperature fluctuates, influencing the metabolism in ectothermic organisms (Hoffmayer et al. 2012). The increase in water temperature reduces the solubility of oxygen in the water, resulting in higher values of lactate concentration (Hoffmayer et al. 2012). Given that nursery grounds are located in shallow waters, the increase in water temperature is more significant, and by adding a stressful event as bycatch, the risk of mortality is higher.

3. Drone technology is particularly useful when sampling small marine areas because they can be launched and landed easily from the boat (Goodman, 2020). During the study, we found that juvenile shark's abundance registered by drones during March, June, October, and November showed that there is a greater abundance during the warm season (March) rather than the cool season (June, October, November). These results agree with those described by Goodman (2020) in which the abundance peak for this species is recognised in late February.

Further, benthic BRUVs technology is a non-invasive tool that revealed a greater presence of juvenile blacktips over other elasmobranch species at Puerto Grande Bay (Figure 1). We have delineated a baseline of abundance and presence of juvenile blacktips at this bay by using both technologies. Further sampling is needed to make conclusions about this component of the research.

In conclusion, our results suggest there is a need for a temporal management strategy during the warm season when the water temperature is higher and there is greater abundance of neonates. This way, by-catch pressure during parturition months will be mitigated, reducing the chance of neonatal mortality because of environmental and anthropogenic stressful events.

Figure 1. Footage taken by benthic BRUVs showing a juvenile blacktip shark at Puerto Grande Bay.

4. Briefly describe the involvement of local communities and how they have benefitted from the project.

During the project execution, local community has participated at different levels regarding the objective's accomplishment.

1. We involved a local master's student, Karen Ascencio, who was trained in juvenile shark handling, including blood extraction at the field. She co-led fieldwork, was trained on data analysis, and published her masters' thesis regarding the results found during this project. Here, I attach the PDF* document where you can find more details on analysis, results, and discussion about this project. Local MSc. Karen Ascencio will be soon publishing her work in an indexed journal.

2. We involved young females from the community (23 - 30 years old) to assist with shark handling and to operate blood field meters. Women were interested in conservation projects, and for some of them this was their first time looking at sharks so closely.

3. We hired Manuel Yépez (an experienced local fisherman) as captain and his crew. Fishermen helped us with local knowledge and experience about juvenile sharks. They told us about how bycatch occurs, and they were really interested on how to mitigate shark mortality during fishing activities.

4. We hired Miss Lupita Revelo, a woman from the fishermen's community, to assist with the daily lunches for the crew. She oversaw preparing to-go lunches for every fieldtrip.

5. We involved local park rangers during fieldtrips; they are the Galapagos National Park representatives and the main authorities on board. Park rangers helped in handling sharks and made sure that all animal handling protocols were authorised and correct.

6. For gillnet repairment, we hired a group of retired fishermen who worked on monofilament sewing to fix all wholes the net had.

5. Are there any plans to continue this work?

Continuing with this work, we are interested in assessing how the anthropogenic activities (fishing, tourism, vessel cleaning) at potential nurseries are affecting the critically endangered scalloped hammerhead shark. We would like to develop a telemetry and genomic study of collected tissue samples to evaluate the connectivity that the different nursery grounds have for this species.

After the experience and skills devolved during the execution of the current project, we think that we are qualified to manipulate hammerheads, which are known to be more delicate species.

We already implemented the nursery ground monitoring into an undergraduate teaching course, so we can cover some expenses. We will also be partnering with

other educational institutes to secure collaboration and partial funding, and we hope to apply to The Rufford Foundation for a third stage of funding. We want to keep developing and expanding this work into other islands of the Galapagos archipelago.

From a larger perspective, the next stage of our research program involves the following key steps.

a) Designate Puerto Grande as a sentinel site and develop a standardised indicator to monitor trends of blacktip and hammerhead shark juveniles for the marine reserve.

b) Use our knowledge obtained from San Cristobal to predict and then ground truth and map other nursery sites across the reserve.

6. How do you plan to share the results of your work with others?

We are seeking to meet with the Galapagos National Park authorities to present results from the first Rufford project and the current one, to suggest measures for an improved management of this key areas. Results of this research will be shared in the 2022 Galapagos Symposium organised by the Galapagos Science Center in collaboration with the Galapagos National Park Directorate. During this event, the fishermen community, national park guides, park rangers, and general public is invited to listen to talks and participate in forums. After symposium occurs, organisers will publish memories from the event which will also be shared. In collaboration with local MSc Karen Ascencio, we will be publishing this work in an indexed journal and will be giving talks to local students from Universidad San Francisco that are pursuing careers in marine research.

7. Timescale: Over what period was the grant used? How does this compare to the anticipated or actual length of the project?

The grant was first used for 5 field days during February 2020 (first update report). After this, the pandemic stopped the use of the grant until March 2021 when fieldtrips restarted, until the end of the season in June 2021. We received additional funding from MigraMar Research Organization to fulfil the benthic BRUVs and drone components carried out during March, June, October and November 2021. The length of the project was longer than that proposed at first. We delated our research activities for one year to restart them in 2021 with all biosecurity and safety protocols.

8. Budget: Provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

Item	Budgeted Amount £	Actual Amount £	Difference £	Comments
MigraMar overhead (10%)	600	600		Expense covered entirely by Rufford grant
Subsistence payment: Accommodation (£300 per month for 3 months)	900	900		Expense covered entirely by Rufford grant
Boat time (£245 per day for 15 days)	3675	4080	+405	Expense covered entirely by Rufford grant. Given increase in gas price, boat time per day was £272 rather than £245 (initially budgeted).
Per diem in field (£33 per day for 15 days for all participants)	495	495		Rufford covered £340 and MigraMar **£155***
Syringes (*100)	40	40		Expense covered entirely by MigraMar
Kayak rent (£40 per day for 3 days)	120		-120	We found that using kayak was not the best method. Rather we snorkeled.
Gillnet repair	210	80	-130	Expense covered entirely by Rufford grant
Heavy net "chinchorro"	400	400		Expense covered entirely by MigraMar
Field meters for blood analysis	880	880		Expense covered entirely by MigraMar
Totals	**7320**	**7395**	**+155***	**We assumed an exchange rate of USD1 equivalent to £0.80**

9. Looking ahead, what do you feel are the important next steps?

Since the first Rufford Small Grant, we have evidenced that Puerto Grande is a key area for the development of different elasmobranch species. We have demonstrated that this bay is a shared habitat for the blacktip shark *Carcharhinus limbatus* and the critically endangered scalloped hammerhead *Sphyrna lewini*, an iconic species from the Galapagos and the Eastern Tropical Pacific region. We would like to retake the capture-mark-recapture study with both species (postponed because of the pandemic). With this information we would be able to estimate the population size and understand the ontogenetic spatial ecology of these two juvenile shark species in a bay impacted by human activities.

As exposed in the results, bycatch is a critical anthropogenic stressor that prejudices shark health, but heavy metals and hydrocarbons (water contamination) produced by touristic and fishing boats is also worrying. From 2016 until 2022, we have seen some signs of skin diseases in some of the sampled juveniles. It would be of our interest to understand how these contaminants are affecting sharks' health, including the sampling of scalloped hammerheads.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

The Rufford Foundation logo was not used yet. We will use the logo soon by the time we present the results in the next 2022 GSC Symposium, as well as in presentations for the Galapagos National Park Directorate and talks for local students.

Recently, we had published a paper in Environmental Biology of Fishes Journal regarding the discovery of Puerto Grande as a putative nursery ground for the scalloped hammerhead shark. The results presented in this publication include those individuals opportunistically sampled during the first and second Rufford Small Grants. Here, we provide a link (https://rdcu.be/cEBdA) where you can find the full-text publication, and you can find the pertinent acknowledgements to Rufford Foundation.

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Alex Hearn
Head of the project, mentoring, and supervision on field activities and data analysis.

Karen Ascencio
Local student. Leaded logistics and co-leaded field activities. Main data analyst.

Yasuní Chiriboga
Leaded logistics and co-leaded field activities. Coordination with fishermen.

Gyslaine Mendoza
Local student. Assistant during field activities.

Carolina Estrada
Local woman from the community. Assistant during field activities.

Jason Castañeda
Park ranger. Supervision on animal handling and assistant during field activities.

Diana Pazmiño
Local professor and researcher at GSC. Assistant during field activities.

Cristina Veintimilla
Marine Lab Coordinator at GSC. Assistant during field activities.

Daniel Armijos
Coordinator of shark research. Assistant during field activities.

12. Any other comments?

Thanks to the support of The Rufford Foundation we were able to start building local capacity in the island and will be able to make a change in favour of a suitable management of nursery grounds for sharks, and as a whole habitat for other elasmobranch and commercial species as well. We hope our project will expand to other islands at the archipelago, and that The Rufford Foundation will continue to support this exciting and essential project in the Galapagos.

13. Bibliography

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