The Rufford Foundation
Final Report

Congratulations on the completion of your project that was supported by The Rufford Foundation.

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. The Final Report must be sent in **word format** and not PDF format or any other format. We understand that projects often do not follow the predicted course but knowledge of your experiences is valuable to us and others who may be undertaking similar work. Please be as honest as you can in answering the questions – remember that negative experiences are just as valuable as positive ones if they help others to learn from them.

Please complete the form in English and be as clear and concise as you can. Please note that the information may be edited for clarity. We will ask for further information if required. If you have any other materials produced by the project, particularly a few relevant photographs, please send these to us separately.

Please submit your final report to jane@rufford.org.

Thank you for your help.

Josh Cole, Grants Director

Grant Recipient Details	
Your name	Eliana Alfaro Córdova
Project title	Characterization of Mobulid ray fishery in Northern Peru through field observation and DNA barcoding techniques
RSG reference	17953-1
Reporting period	August 2015 – August 2016
Amount of grant	£5000
Your email address	elianaalfarocordova@gmail.com
Date of this report	16th August 2016

1. Please 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
Barcode sequences for mobulid rays occupying the marine habitats in northern Peru			x	We sequenced 102 high quality samples of mobulids for analysis. We obtained sequences of all mobulids species reported for Peru: *Manta birostris* (n=5), *Mobula munkiana* (n=39), *Mobula japanica* (n=11), *Mobula thurstoni* (n=32) and *Mobula tarapacana* (n=14).
Describe the composition of mobulid species caught by the gillnet fishery of Chiclayo and Zorritos.			x	We registered captures of all *Mobula* species through onboard and shore based observations. The main species caught was *M.japanica*, followed by *M.thurstoni*. No *Manta birostris* was reported neither captured nor landed by gillnet fisheries.
Identify the main fishing grounds where mobulids have been captured.				We identified the main fishing grounds of those species, mainly near the coast over the continental shelf.
Inform fishermen from Chiclayo and Zorritos, about the mobulid species that they are catching. Conduct eight training and awareness workshops for fishermen to improve their abilities in morphological identification of mobulids, as well as about the importance of conserving these				We conducted 12 interactive workshops, where we transmitted the awareness about manta and devil rays, and the necessity to improve our knowledge about these species. We also trained fishermen and some local people (women and students) on morphological identification of mobulids, and distributed identification guides and educational material. We verified the improvement on mobulid identification from fishermen at the

species				end of the project. We correctly identified mobulid species (99%) by comparing morphological and genomic identification.
Prove barcoding as a useful tool for other ray and skate species identification.				We demonstrated barcoding as a useful method to verify morphological identification of mobulids at species level. Identification doubts were solved and misidentification was corrected.

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

- Interaction with local people and fishermen. At the beginning of the project interaction with fishermen and local people was difficult. Workshops as well as shore-based and on-board observations did not cover our expectations. During workshops audience participation was low, especially when the group was big, and the acceptance of fishermen to receiving an observer on the boat was not easy to obtain. To solve these problems we started visiting fishermen and their families at home, and realised the following: (1) It was easier since we could cover more fishermen by locating them separately, without the need of finding one day when all of them were available; (2) It was more efficient because we could transmit the message not only to the fishermen but also to the rest of the families (women and children); and (3) The audience was much more confident and talked more, telling us details about mobulid fisheries in the past and in the present, and showed a better understanding of mobulid species identification.

The first and last workshops were developed including the whole group of fishermen, while the four workshops held in between were conducted with small groups (i.e. fishermen and their families). At the end, we identified much more conversation, questions, interaction and opinions about the issue in hand and the project's objectives and results.

- Less trips than expected. We got information from 50 fishing trips, although we expected to 56 fishing trips in total (three trips x 6 months x two ports). Fewer trips were monitored at the beginning because some fishermen took some time to accept our presence on-board. During the last three months of the project (April- - June 2016), pelagic fish were not available and fishermen from San Jose decided to switch gears, from superficial to deep gillnet. Therefore we were not able to get any

on board information on mobulid fisheries during June 2016. Despite the problem was not solved, we consider it was not significant for the results since we approached 89% of the objective.

- **Morphological identification**. During the first period of the project (first 4 months), identification of mobulids at species level was quite difficult for on-board observers. Even though observers attempted to take pictures from all sampled animals, not all captured mobulids had pictures to verify the "correct morphological identification". During four trips we were only able to identify captured specimens at genus level (*Manta* or *Mobula*). We solved this problem by training observers through workshops. We also designed and printed two identification guides to be used on-board.

- **Collection and preservation of samples.** Sampling for DNA analyses is quite problematic when field conditions are not good. In this case, samples were collected even on-board or at the beach where fishermen arrived to sell the fish. It was a problem sampling at the beach, with very short time (fishermen wanted to sell the fish as soon as possible), wind, sun and sand. Preservation of samples at -20 °C was another problem, since these small villages do not usually have electricity or a freezer. To solve these problems we incorporated an extra step for sampling. First, the sample was stored in salt, and then it was washed with distillate water to get a subsample (from the less degraded part), which was preserved in ethanol at 80% at -20°. Nevertheless, 70% of all collected samples were analysed properly (correct DNA extraction and sequencing).

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

I. Increased awareness and knowledge about mobulids in Peru and their importance.

At the end of the project 12 workshops (six per port) were conducted alongside local people, with emphasis on fishermen. We applied dynamic talks, where the audience was able to identify the importance of mobulids as key components of marine ecosystems, as well as the problem of mobulids overfishing and general information about the species.

Additionally, we were invited to two interviews to talk about the project at San Jose's local radio. In order to share information about mobulids to more local people from the villages, we designed and printed two identification guides with relevant information on mobulids in Peru, as well as educational material for children (Annex 2). We noticed that the perception on mobulids changed on both groups of people (i.e. both villages). Through tests on species identification at the beginning and end of the project, we noticed an improvement of 80% in San Jose and 50% in Zorritos.

Although the project has concluded, communication with local people from San Jose and Zorritos remains.

II. **Description on mobulid captures**

50 fishing trips and 193 sets were monitored through on-board observations. 31% of the sets registered mobulid captures. We calculated the nominal CPUE per set, based on net length (km) and set duration (day). Despite the mentioned difficulties on mobulid identification at species level during the first months of the study, we found that the main captured species was *Mobula japonica* (cpue 1.6 ±1.65), followed by *Mobulid spp* (cpue 1.49 ± 0.77) and *Mobula thurstoni* (cpue *0.36* ± 0.11).

No captures for *Mobula munkiana*, *Mobula tarapacana* or *Manta birostris* have been reported by on-board observations during the project.
Every set was geographically localised and mapped. We could identify the main fishing grounds where mobulids were captured during the study. These zones were near the coast over the continental shelf. Mobulid catches also showed a temporal trend, increasing between September 2015 and February 2016, with a peak in October 2015 (9.6 ± 11.2 mobulids [km/day]-1).

518 caught specimens of *M.japanica* were measured and sexed. We could identify the main specimens were juvenile with Disc Width (DW) less than 176 cm (Notarbartolo-di-Sciara, 1988).

Results suggest sizeable mobulid captures in Zorritos and San Jose, which could reflect an opportunistic behaviour of fishermen who retain mobulids when target species are not available, to be sold as meat in local markets.

Shore-based Observations of Mobulid landings

Shore-based observers were deployed in San Jose and in Zorritos. Data on the total number of mobulids landed per vessel was collected daily between August 2015 and February 2016. We registered 869 *M.japanica* landed by 16 gillnet vessels in San Jose, while in Zorritos 833 *M.japanica* and 177 *M.thurstoni* were registered landed by 20 gillnet vessels. The highest values for mobulid landings were registered during October. In most of the cases only the pectoral fins of mobulids were landed (no head), so we counted right fins.

Trade of mobulid meat was observed in both ports. Prices per kilogram fluctuated between 2.5 and 4 Nuevos Soles (0.75 – 1.20 US dollars) in both ports, depending upon the colour of the meat (white meat has higher prices than grey) and the other species available for purchase. Mobulid meat from Zorritos was sold mainly in the

city of Chiclayo (San Jose Province) while mobulid meat from San Jose was sold in local markets. We also became aware of an apparent cross-boundary market of mobulid meat between Peru and Ecuador, but we did not investigate this in detail as it was beyond the scope of the project.

DNA sequencing of mobulid species and verification of morphological identification

We collected 156 samples of mobulids during on-board observations, shore-based observations (considering other gears) and additional visits to local markets. One picture of each sampled specimen was taken as a registration of morphological identification. Samples were collected with a sterilised dissection kit, and stored with salt or 70% ethanol while stay at sampling place. Once samples arrived to the lab, we washed them with distillate water, collect a subsample from the central part of the tissue (to avoid contamination from the field) and stored in a labelled vial with 70% Ethanol at -20°C avoiding direct light exposure.

A total genomic DNA from each sample were extracted using DNAeasy (Qiagen) and by following the manufacturer instructions. Approximately 650basepairs of the gene cytochrome oxidase 1 (COI) from the mitochondrial DNA were amplified through a polymerase chain reaction (PCR) and using the universal primers FishF2 (59TCGACTAATCATAAAGATATCGGCAC39) and FishR2 (59ACTTCAGGGTGACCGAAGAATCAGAA39). PCR products were run by electrophoresis to confirm amplification. 138 samples were amplified at high quality. The forward and reverse strands were purified and Sanger-sequenced by Macrogen USA. The sequences obtained were edited using the software Sequencher 5.4.5. In order to identify the species for each sample the resultant sequences were input into the Barcode of Life Data Systems. 102 samples were sequenced at high quality to be analysed. We obtained sequences for the five species of mobulids reported in Peru: *Manta birostris* (n=5), *Mobula munkiana* (n=39), *Mobula japanica* (n=11), *Mobula thurstoni* (n=33) and *Mobula tarapacana* (n=14). The high level of degradation (26% samples with DNA extraction) could be due to contamination during sampling or an inadequate storage method.

Morphological identification was correct at 100% for *Manta birostris* and *Mobula tarapacana*, 95% for *Mobula munkiana*, 93% for *Mobula thurstoni*, and 67% for *Mobula japanica*. Two specimens with doubts identification were identified through their barcoding.

Errors in mobulid identification occurred mostly during shore-based observations, where observers had to work with incomplete bodies, using only colour patterns of pectoral fins to identify mobulid species (no head, nor tail). The major problems on species identification observed were between *M.japanica*, *M.munkiana* and

M.thurstoni. It was difficult to identify specimens of *M.tarapacana* and *M.birostris* based on fins, but we obtained positive results. However it is important to consider all *M.birostris* sampled were landed in Zorritos and all *M.tarapacana* were landed in San Jose.

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

Through the project we improved our relationship with local communities of San Jose and Zorritos. On December 31st 2015, a new Peruvian regulation on the conservation of *Manta birostris* was applied. Through the norm, a ban on *M. birostris* fishery was established along National jurisdictional waters (RM N°441-2015-PRODUCE). In this sense, appropriate identification of mobulid species by fishermen, consumers and inspectors becomes even more necessary. Local people from San Jose and Zorritos expressed their awareness on this ban, and asked for more informative tools on morphological identification of mobulids.

Outcomes of the study were shared with local people from the two villages. The audience (fishermen and their families) identified new questions related to mobulids and their ecology, considered important to understand or propose new regulations on their fisheries.

5. Are there any plans to continue this work?

Yes, we are still working in these localities. Communication with fishermen and local people remains. We visit these places periodically and maintain communication by phone. Fishermen are still calling or sharing their pictures through the Internet social-media (i.e. Facebook, WhatsApp) to tell us about information they consider relevant for our study. Because we identified mobulid catches as opportunistic measures by fishermen when target fish are not available, it is important to consider the potential risk that conventional fisheries represent to mobulids. Since *Manta birostris* is the unique mobulid species protected by Peruvian law, not being the case of Ecuador where all mobulid species are protected, we propose improving the knowledge about populations, fisheries and markets of Mobula species at a regional level (including Chile, Peru and Ecuador).

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

We already shared the outcome of the project at a public closing meeting, where the proposals and results were shared with the San Jose and Zorritos communities. During these meetings new collective ideas have been proposed, which we expect could be achievable soon.

Concerning the scientific community, we have already summited an abstract about mobulid captures, to be presented during the V Colombian Meeting on Chondrichthyes (http://encuentro2016.squalus.org/), and will present results on captures and species identification through DNA sequencing in the following V Peruvian Congress on Marine Science (http://www.concimarperu.com/).

Additionally we expect to submit two scientific articles on the project outcomes in an international scientific magazine.

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

The RSG was used consecutively along the year of the project. However, the main expenses were made at the beginning and end of the project. Purchase of sampling material and kit for DNA extraction and sequencing, as well as training workshops were done during the first three months. Monthly expenses were made for on-board observers trips, workshops, and sampling. Identification guides for adults and kids were designed and printed at the middle phase of the project. Lab work was conducted from January to June and expenses for this service were made in two phases. In July DNA sequencing service was paid.

We consider that the money was spent according to what was anticipated in the project. However, an unexpected problem during the shipping of the samples to Macrogen (USA) delayed the last phase of the project, and we started the analyses of DNA sequences at the end of July.

8. Budget: Please 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. 1 £ sterling = 4.44 Nuevo Sol

Item	Budgeted Amount	Actual Amount	Difference	Comments
Field Observation: Training (3 talks per port)	315	200	115	We expected three workshops of 3 hr per port. We conducted one full day workshop per port.
Field Observation: On-board observations (3 per month per port during 6 months)	1800	1300	500	We could not cover the amount of trips expected, due to weather anomalies and changes of fishing gear in some boats.

Field Observation: Observations on port (daily observations per port during 10 months)	3000	3000	0	
DNA barcoding: Sampling (materials)	600	600	0	
DNA barcoding: Sample transportation from field to lab (Chiclayo/Zorritos to Lima)	700	700	0	
DNA barcoding: Analysis in a genetic DNAeasy, primers, electrophoresis)	638	1837	-1199	Costs lab (lab costs, of the lab raised.
DNA barcoding: DNA sequencing (2 chains for 60 samples)	750	1019	-269	We sequenced more samples than expected in order to reduce probabilities of insufficient data for some species
DNA barcoding: Sending costs to Macrogen	62	47.23	14.77	
Workshops: Travel	490	400	90	We used cheap tickets costs (tickets, viatics) (on sale)
Workshops: Material (15 participants per workshop)	600	400	200	We printed all material together saving expenses for small amounts.
Workshops: Rent of meeting place	60	60	0	
Total	9015	9563.23		

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

Considering the new global conservation tendencies of mobulids' ecology and fisheries, which propose including all *Mobula* species in Appendix II CITES, and taking

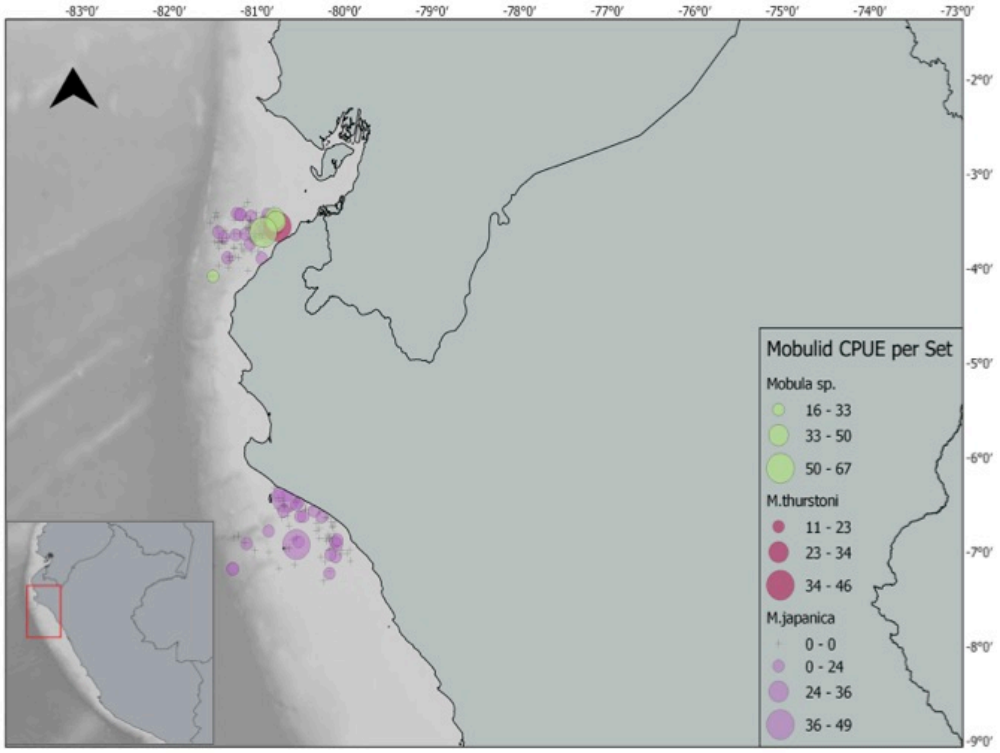
into account the regional PAN (Elasmobrach action plan) for Southeast Pacific, it is important to develop further studies to better understand those species poor known such as *Mobula tarapacana* (recently listed as vulnerable by the IUCN Red List). Mobulids are migratory species with broad distribution and displacement; in Peru only *Manta birostris* is regulated (banned by law) while in Ecuador and Chile all mobulid species are banned. In this sense, collaborative research is urgent at a regional level to implement efficient management plans.

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

Yes, each workshop was developed using power point presentations in which the RSGF logo was used. Additionally, two identification guides (one in black and white and other in colour), and one education material, were made using the RSGF logo.

11. Any other comments?

Taking into account that only *Manta birostris* is regulated by Peruvian legislation, it is important to evaluate the correct way to identify between mobulid species during inspections on landing zones. Despite this project showed correct morphological identification, this only could be possible though training workshops. On the other hand, difficulties on mobulids identification were also described through the project, recognizing DNA barcoding as a useful tool to clarify doubts. However, it is important to develop further studies to find better sampling and storage methods to avoid degradation of samples.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Mobulid CPUE per Set
Mobula sp.
16 - 33
33 - 50
50 - 67
M.thurstoni
11 - 23
23 - 34
34 - 46
M.japanica
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24 - 36
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