

Project Update: March 2017

The anticipated length of the project was 30 months. To date, the RSG was used to cover about one-third of the costs foreseen in the work plan. Our work will continue for another 18 months with grant provided by RSG.

Objectives and outcomes so far:

Field expeditions – Achieved

Forty-one expeditions were performed from February 2016 to January 2017 along 355 km of coast, between Lagoa do Peixe (31°21'S; 051°02'W) and Chuí (33°44'S; 053°22'W), Rio Grande do Sul. During these expeditions, humeri and tissue samples were collected from 61 stranded dead loggerhead turtles, ranging from the small size of 11 cm curved carapace length (CCL) up to a maximum of 114 cm CCL. These expeditions were carried out with NEMA support.

Samples from partners – Achieved

The NGOs NEMA and Tamar Project provided tissue samples for DNA analysis from loggerheads caught in longline (n=245), trawling (n=55) and fishing weirs (n=75). Furthermore, Nema and Tamar Project also provided additional humeri and tissue samples from turtles washed ashore along Brazilian coast, bringing the number of individuals sampled to a total of 123.

DNA extraction, PCR reactions, sequencing and mtDNA haplotype classification – Partially achieved

To date, from the 378 samples collected in fishing activities, 311 were processed and 172 were sequenced. Sequences (~740 bp) were classified according to the previously described mtDNA haplotypes. Remaining samples will be sent to sequencing on March 7th and 31st. After that, it is planned to start the processing of DNA samples from loggerheads stranding.

Histological humeri processing – Partially achieved

Humeri began to be processed, as set out in project schedule, to estimate age and growth rates of loggerhead sea turtles (see Photo 1 and Photo 2). Histological processing started with humeri from individuals with CCL less than 70 cm, which are able to present the first growth line (*annulus*), which are essential to fit growth models.

Stable isotopes analysis – Not achieved

Stable isotope analysis of growth lines will occur after histological humeri processing. Collection of growth lines is expected to start in June 2017.

Microsatellite analysis – Not achieved

Since most samples provided by partners were in advanced state of decomposition, we chose to do microsatellite analysis after all DNA extractions, in order to select those samples with better DNA and to avoid repetitions in the genotyping, which would significantly increase the cost of analysis.

Preparation and submission of scientific papers – Partially achieved

The first scientific article foreseen in this project is already under preparation and received the provisional title "Genetic composition of mixed stocks of loggerhead sea turtles caught in fisheries in Southwestern Atlantic Ocean" and will be submitted to *Conservation Genetics* journal up to December 2017.

Although this project is still ongoing, preliminary results have provided valuable information for the conservation of loggerheads populations occurring in the Southwestern Atlantic Ocean (SWA). The most important outcomes are described as follows:

- SWA represents an important foraging and development ground of loggerhead sea turtles at different life stages, and also holds extensive commercial fisheries. Incidental mortality in fisheries is the most important threat to sea turtles in Brazil and causes the decline of several loggerhead turtle populations. To date, seven haplotypes were found among loggerheads sea turtles caught in fisheries in three distinct foraging aggregations along Brazilian waters: CC-A2.1 (7%, n=8), CC-A3.1 (1%, n=1), CC-A4.1 (31%, n=34), CC-A4.2 (47%, n=52), CC-A11.6 (4%, n=4), CP1.1 (3%, n=3), and one new haplotype (5%, n=6). Sequences were classified according to the previously described mtDNA haplotypes from nesting sites around the globe. The presence of a new Pacific haplotype in our samples indicate that there still are nesting sites which genetic composition remain unknown and, consequently, that Pacific populations requires further studies to ensure populations assessment and to identify more management units. The occurrence of these seven haplotypes indicates that SWA serves as foraging ground for populations from several rookeries around de globe, mainly from Brazil, western and eastern North Atlantic, Mediterranean Sea and Pacific Ocean. These data also supports the high migratory behaviour of sea turtles and highlight that bycatch in fisheries raises concern over the population structure and genetic diversity of loggerhead sea turtles. Thus, our work will provide valuable information for the long-term conservation of endangered loggerhead populations by identifying the genetic composition of mixed stocks and possible losses of genetic diversity, which will supply concise information to fisheries management plans (e.g. time-area closures) or to define marine conservation units;
- In addition, two individuals were identified as loggerhead-olive ridley hybrids (i.e. they presented the morphology of pure loggerhead, but their mtDNA was characteristic to olive ridley turtle *Lepidochelys olivacea*). These data point out to another conservation issue. In Brazil, sea turtle breeding groups present high hybridisation rates (~43%), which is concerning due to the possibility of sterility, lower fitness/survival of hybrids or advantage of hybrids over pure turtle species, increasing pressure by competition in feeding areas and nesting sites. The causes behind hybridisation in Brazilian territory remain unclear and require further investigations. The outcomes of this work will contribute towards a better understanding of this process and can be contribute to analyse negative impacts of this phenomenon;
- The development of this project is also contributing towards the implementation of the National Action Plan for the Conservation of Sea Turtles, which is part of the

public policies for environmental conservation in Brazil. Our work are covering actions proposed in, as follow: (1) monitoring the main fisheries that interact with sea turtles; (2) protecting and monitoring the main foraging grounds, which includes (i) continuing research and conservation activities in priority areas, and (ii) continue monitoring stranding in relevant sea turtle occurrence areas and investigate the causes of these events; (3) increasing the scientific knowledge related to the conservation of sea turtles, which includes (i) information of reminiscent genetic diversity, population structure and gene flow (migration routes) throughout sea turtle populations, and (ii) complementary studies to assess population structure (e.g. through stable isotopes analysis).

Several unforeseen difficulties arose throughout the project, causing delays in the original proposed timeframe:

- Primers deliveries were significantly delayed and therefore caused setbacks in commencing lab work.
- Most samples from loggerheads caught in longline fisheries (~40%), that was provided by partners, were in advanced state of decomposition and it was necessary to perform many PCR repetitions and test different extraction methods in order to obtain higher DNA quality, which took a considerable amount of time.
- In September 2016, two laboratory equipments damaged (water bath and centrifuge). They are necessary for the extraction and purification of DNA. The equipments were sent to repair and returned to the laboratory in January of this year. During this period, laboratory work was very committed.

Future plans:

This detailed report represents the first 12 months of work. As it is possible to observe in the RSG 18836-1 project, the original timescale is 30 months and it will be necessary another 18 months of work to reach the proposed objectives.

We will communicate results in conferences and will submit three papers to high-impact journals. Our working progress is also being shared with Chico Mendes Institute for Conservation of Biodiversity, responsible for the conservation of endangered species in Brazil. Our team and partner NGOs (e.g. Tamar Project, whose coordinator Maria Marcovaldi is the regional vice-chair of the IUCN Marine Turtle Specialist Group, member of the Inter-American Convention for the Protection and Conservation of Sea Turtles, and editor of Tamar Newsletter) also guarantee reporting of the attained results to relevant groups.

The first necessary step is to conclude histological processing of humerus from loggerheads and amplify their respective mtDNA. After that, we will perform de collection of growth lines of each humeri processed. By combining molecular techniques with stable isotopes analysis and skeleton-chronology, this work will provide the first insights on how the genetic characteristics of populations are related to foraging, habitat use and growth dynamics. The amplification of nuclear markers is also an important next step to improving our

understanding of loggerhead populations by identifying bottleneck events, i.e. possible losses of genetic diversity due to population decreases caused by human activities such as fisheries. This activity is expected to start in the second half of this year.

The RSGF receive publicity during the Biological Oceanography Meeting that was promoted by Rio Grande Federal University- FURG in October 2016. In this meeting it was exposed our work plan and the partial results about loggerhead sea turtles caught in fisheries. The same occurred on February 20th, at the Marine Megafauna Ecology course promoted in FURG for students from the province of Ontario, Canada (Photo 3 and 4). The course was offered by the University of Waterloo through the Field Biology Program of the Universities of Ontario. Also, we are reporting our work progress to Chico Mendes Institute for Conservation of Biodiversity, responsible for the conservation of endangered species in Brazil, and to NGOs NEMA and Tamar Project.

I would like to express my gratitude to the RSGF for the financial support. Loggerhead sea turtles are suffering numerous threats at the SWA and the scarce knowledge of population structure makes difficult the implementation of effective conservation measures. An example of this is the implementation of Albardão National Park, in southernmost Brazil, that was proposed in 2008 and until now has not been accepted by the governmental authorities. This proposed conservation unit includes the most important archaeological site of the Brazilian coast and serves as an important foraging and development ground of many endangered and critically endangered marine species, such as the Franciscana dolphin (*Pontoporia blainvillei*), angel sharks (*Squatina* spp.), Brazilian guitarfish (*Rhinobatos horkelii*), oil shark (*Galeorhinus galeus*), hammerhead sharks (*Sphyrna* spp.), as well as leatherback (*Dermochelys coriacea*) and loggerhead sea turtles. We are sure that the results of our work will be crucial for the implementation of this conservation unit and that they will also promote a wide application in marine conservation along SWA. Without Rufford Small Grants, it would be more difficult to achieve these conservation goals and to increase our knowledge about sea turtles populations.

