

## The Rufford Small Grants Foundation

### Final Report

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

We ask all grant recipients to complete a Final Report Form that helps us to gauge the success of our grant giving. 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. 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](mailto:jane@rufford.org).

Thank you for your help.

**Josh Cole, Grants Director**

#### Grant Recipient Details

<b>Your name</b>	Maira Carneiro Proietti
<b>Project title</b>	Genetic diversity and structure, mixed stock analysis, and migratory patterns of hawksbill turtles ( <i>Eretmochelys imbricata</i> ) feeding around northeast Brazil
<b>RSG reference</b>	8110-2
<b>Reporting period</b>	11/2010 to 11/2012
<b>Amount of grant</b>	£ 4965
<b>Your email address</b>	<a href="mailto:mairaproietti@gmail.com">mairaproietti@gmail.com</a>
<b>Date of this report</b>	13 November 2012

**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
Sample collection			X	We performed seven expeditions for skin sampling at São Pedro and São Paulo Archipelago, Abrolhos National Marine Park, and Arvoredo Marine Biological Reserve, and obtained a satisfying number of samples (n = 84). The NGOs NEMA and Project Tamar provided additional samples from the coast of Bahia, Ceará and Rio Grande do Sul states (n = 103), bringing the number of samples to a total of 187.
DNA extraction, PCR reactions and sequencing			X	Of the 187 samples, 157 presented sufficient DNA for amplification (PCR) and sequencing. The remaining 30 samples were excessively degraded and resulted in no or low-quality DNA that did not amplify through PCR.
mtDNA haplotype classification			X	740bp sequences were classified according to the previously described mtDNA haplotypes (n=137).
Compilation of available genetic data for Atlantic hawksbills			X	Data was compiled for a total of 17 mixed stocks and 12 rookeries.
Evaluation of genetic diversity			X	We evaluated standard genetic diversity parameters (haplotype and nucleotide diversities) for the study areas.
Analysis of population structure			X	We performed genetic differentiation tests (standard F-statistics) for comparison of the areas.
Evaluation of natal origins			X	Probable natal origins of immature animals at the study areas were estimated in a Bayesian framework, through many-to-many mixed stock analysis.
Analysis of ocean drifters as a proxy for sea turtle dispersal		X		This is currently being performed for comparison with natal origin results.
Microsatellite analysis	X			We chose to remove microsatellite analysis the project, since this molecular marker would significantly

				increase costs and would not provide relevant information for completing our main objectives.
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**2. Please explain any unforeseen difficulties that arose during the project and how these were tackled (if relevant).**

Several unforeseen difficulties arose throughout the project, causing delays in the original proposed timeframe. Expeditions were carried out for a longer period than expected (over 1 year) in order to obtain a satisfactory sample number. Due to shortages in stocks from suppliers, some laboratorial material deliveries were significantly delayed and therefore caused setbacks in commencing lab work. Since most samples provided by partners were in advanced state of decomposition, it was necessary to perform many repetitions and test different extraction methods in order to obtain higher DNA yields, which took a considerable amount of time. Polymerase Chain Reaction amplifications also had to be performed several times to improve product quality, since DNA sequencing requires very concentrated and pure DNA. Due to the necessary repetition of PCRs, we had several contamination issues, and many stock materials, reagents and primers had to be successively replaced. The following step, DNA sequencing, was performed through a molecular biology company that took a large amount of time to deliver results. Sequencing is a sensitive reaction and some sequences did not present sufficient clarity, having to be redone. Haplotype identification also proved to be a challenge, since there are no organised databases available and literature reports haplotypes of different lengths and names, with no straightforward classification and many sequences unavailable in the online genetic database GenBank (<http://www.ncbi.nlm.nih.gov/genbank/>). Obtaining and organising the correct haplotype classification was possible only through many correspondences with other researchers. All these factors caused significant delay in obtaining results.

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

We obtained several important results during this project; the three most important outcomes are described as follows:

1) *Identification of important feeding/development areas for immature hawksbills in Northeast Brazil.* Hawksbill turtles have suffered drastic population declines, rendering this species a Critically Endangered classification in the World Conservation Union Red List (IUCN Red List) and in Appendix I of the Convention for the International Trade of Endangered Species of Fauna and Flora (CITES). In Brazil, hawksbills nest mainly at two rookeries (located at Bahia and Rio Grande do Norte states), which have been studied to some extent but major gaps exist in the knowledge of immature hawksbill turtles that use Brazilian waters for feeding and development. By describing the occurrence of hawksbills at the São Pedro and São Paulo (SPSP) Archipelago, Abrolhos Marine National Park and Arvoredo Marine Biological Reserve, through in-water visual census and observations, tagging and biometrics (see Figure 1 for photographs of field activities), we attempted to address part of this gap. We identified SPSP Archipelago and Abrolhos Park as two high-use areas for immature animals of this species, and gathered interesting information on habitat use, animal sizes, permanence at areas (registered through tagging and photographic identification), feeding habits, and cleaning relations with reef fish (see Figure 2 for examples of in-water observations). This information is essential for understanding hawksbill populations and their ecological roles within their habitats, and this work demonstrated that Brazil hosts important hawksbill turtle foraging

grounds, which should be preserved for the recovery of *E. imbricata* populations. More detailed results and comments on this outcome can be seen in issue 135 of Marine Turtle Newsletter: <http://www.seaturtle.org/mtn/archives/mtn135/mtn135p4.shtml>

2) *mtDNA characterization, genetic diversity, and population structure of hawksbill feeding areas along the Brazilian coast.* Genetic studies are important tools for understanding connectivity and structure of endangered populations, and Brazil lacks adequate genetic characterization of its immature hawksbills. In this project we analysed DNA in 157 skin samples from immature hawksbills from the following study areas: São Pedro and São Paulo Archipelago, coastal Ceará state, coastal Bahia state, Abrolhos National Marine Park, Arvoredo Marine Biological Reserve, and Cassino Beach (the last two were grouped into “South Brazil” due to small sample size and geographic proximity). Locations of study areas can be seen in the attached Figure 3. We encountered ten distinct 740bp mtDNA haplotypes at our areas; haplotype frequencies for the study areas can also be seen in Figure 3. We highlight the high occurrence (almost 80%) of haplotype A01, followed by a frequency of approximately 8% of A62, and less than 4% for all other haplotypes. We also highlight the presence of a previously undescribed haplotype (A92, observed at SPSP) and the occurrence of four BR3 haplotypes (three in South Brazil and one at the coast of Ceará). This last haplotype is actually characteristic of loggerhead turtles (*Caretta caretta*), and after confirmation that the all animals that had this haplotype presented hawksbill turtle morphology, we conclude that they were *Eretmochelys imbricata* x *Caretta caretta* hybrids. In Brazil, the only other observation of *Ei* x *Cc* hybrids has been of adult females nesting at the Bahia rookery, and this is an indication that hybrids born at Bahia migrate to these feeding grounds. Genetic diversities were average, with SPSP presenting highest haplotype diversity (0.64), followed by South Brazil (0.56), Bahia coast (0.47), Abrolhos (0.26), and Ceará coast (0.25). Although other studied hawksbill feeding grounds commonly present higher haplotype diversities (around 0.6), our lower haplotype diversities could be a reflexion of small sample sizes at individual areas, hindering the detection of rarer haplotypes. Another possible explanation is that the majority of other studied feeding grounds are located in the Caribbean region, where most hawksbill rookeries are located within the Atlantic basin. Therefore, these feeding grounds could be composed of individuals from a larger number of rookeries, resulting in higher diversity indexes. The Brazilian study areas did not present overall genetic differentiation among them, but were significantly different from feeding grounds located in Western Africa and the Caribbean, indicating a regional structure in hawksbill feeding populations within the Atlantic.

3) *Estimations of natal origins of animals.* The genetic characteristics of immature hawksbills were used to estimate the natal origins of these feeding populations, through many-to-many mixed stock analysis. Results indicate that the studied feeding populations are composed mainly of individuals from the two main rookeries in Brazil: Forte Beach, in Bahia state, with around 500 females nesting annually; and Pipa Beach, in Rio Grande do Norte state, with around 100 annual female nesters. Estimated contributions from these rookeries to the study areas were similar, with Forte Beach contributing between 10-35%, and Pipa Beach between 12-40%. Other possible contributors include some Caribbean rookeries, with Barbados, Antigua and Barbuda, and Cuba presenting contributions of approximately 10% to some of the study areas. African rookeries were not shown to contribute to the studied feeding grounds, but this again could be due to our small sample sizes that fail to detect the rarer EATL (Eastern Atlantic) haplotype, characteristic of West African rookeries. Understanding the origins of animals at their non-reproductive stages is extremely important for determining how impacts can be shared by seemingly separate populations that are in actuality connected through migration. This is of special significance for the hawksbill turtle, since the once widespread hawksbill shell commerce is continuously subject to debate by CITES, and effective conservation and/or

sustainable use strategies must be based on how populations connect. Although the main origins of the studied immature hawksbills were determined to be rookeries located in Brazil, avoiding problems inherent to trans-boundary migrations, some of the areas are located thousands of kilometres from these rookeries. The identification of contributions from the Caribbean is also significant and should be addressed when considering the management of these feeding populations.

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

Although this project did not foresee direct involvement of local communities, there was involvement of some local people. At Arvoredo Island and Abrolhos Park, we were in direct contact with Navy officials, as well as their families, who were responsible for maintenance and operation of the lighthouses. They accompanied the work and were able to participate in activities, and were always very eager to learn more about sea turtles and their conservation. At these areas, educational activities were also performed on diving boats, and on two occasions at Abrolhos, the local coastal community was taken for a tour that involved sea turtle activities. At the São Pedro and São Paulo Archipelago, transport is provided by a fishing vessel and research is conducted with total assistance from the fishermen. This has been a very interesting exchange, with the fisherman sharing their extensive knowledge of the area, while also demonstrating interest in finding out more about the biology and behaviour of sea turtles.

**5. Are there any plans to continue this work?**

Yes, this work is being continued in several aspects. We are currently evaluating if ocean surface drifters corroborate possible dispersal patterns from natal areas identified by mixed stock analysis. Also, carapace samples from immature hawksbills were collected at São Pedro and São Paulo, Abrolhos and South Brazil, and are currently being processed for analysis in order to better understand the life histories of these animals. This will be achieved through analysis of stable isotope ratios ( $C_{13}:C_{12}$ ,  $N_{15}:N_{14}$ ,  $O_{18}:O_{16}$ ) in successive carapace layers, and using differences in these ratios to evaluate trophic level and habitat use throughout their life cycles. We also plan to return to the Abrolhos Park, and possibly the São Pedro and São Paulo Archipelago, to conclude the initiated surveys for including this data in the Project Tamar (main project acting in sea turtle conservation in Brazil) action plan for the conservation of hawksbills in Brazil, as well as for local management strategies at the Abrolhos Park.

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

The results have started to be shared through scientific conferences and partnerships with other researcher and projects. We have published one scientific paper and are currently preparing two additional papers. Results are being integrated in the Project Tamar database and are very important for formulating next steps in the national action plan for the conservation of hawksbills in Brazil. This information has also been shared with the coordinators of the National Marine Protected Areas (Abrolhos and Arvoredo), and will be considered in the local management strategies. Finally, information on the sea turtles that occur at the São Pedro and São Paulo Archipelago is being shared with the managers of the area, and could play an important part in giving this Archipelago a protected status.

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

The anticipated length of the project was 14 months, but the RSG was used over almost two years due to the previously described difficulties.

**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.**

Item	Budgeted Amount	Actual Amount	Difference	Comments
Expedition costs (travel and food supplies)	£2952	£3460	£508	An additional expedition increased field costs
Absolute ethanol	£31	£31	-	-
Eppendorfs	£23	£250	£227	Additional eppendorfs were necessary for repeating extractions and PCRs
Sealing parafilm	£25	£25	-	-
Biopsy punches	£145	£45	£100(+)	We chose to use scalpels instead of biopsy punches to reduce costs
Primers for PCR	£135	£170	£35	Additional primer sets were ordered due to contamination
Taq polymerase for PCR	£230	£460	£230	More Taq polymerase was ordered for additional PCRs
PCR purification kit	£385	£385	-	-
Sequencing reactions	£1039	£1358	£319	Additional sequencing reactions slightly increased costs
<b>TOTAL</b>	<b>£4965</b>	<b>£6184</b>	<b>£1219</b>	Approximate exchange rate for period of expenditures: 1£ = 2.98BRR

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

The first necessary step is to conclude drifter analysis and publish the full results from genetic and ocean currents analyses in a scientific journal. This is currently being worked on and we plan to submit by the end of this year. The analysis of stable isotopes in carapace samples is also an important next step for improving our understanding of the life histories of hawksbill turtles that forage along the Brazilian coast. Another important step is to continue genetic description of sea turtles in Brazilian waters, for a more complete identification of genetically distinct populations and natal origins of these animals. Finally, continuing the exchange of information with other researchers, conservation projects and managers of protected areas is essential for ensuring that

effective measures for the conservation of these endangered animals will be adopted based on an adequate biological and ecological foundation.

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

The RSGF was acknowledged in all produced materials. This included four conference abstracts, which were presented in three posters and one talk, and one paper published in Marine Turtle Newsletter (<http://www.seaturtle.org/mtn/archives/mtn135/mtn135p4.shtml>). The RSGF will be acknowledged in all forthcoming publications involving this project.

**11. Any other comments?**

I wish to express my gratitude to the RSGF for the financial support, and for understanding that unforeseen setbacks were encountered and we were therefore unable to conclude the project in the original proposed timeframe.