# 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** | Luciana Medeiros Silva |
| **Project title** | Population structure and trophic ecology of loggerhead sea turtles *Caretta caretta* in the southwestern Atlantic Ocean |
| **RSG reference** | 18836-1 |
| **Reporting period** | February 2016 to June 2019 |
| **Amount of grant** | £ 4951 |
| **Your email address** | lumedeiros.silva@gmail.com |
| **Date of this report** | 05 June 2019 |

**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 |
|---|---|---|---|---|
| To estimate origin and population structure of loggerhead feeding grounds in the SWA | | | ▨ | In this project, we processed a total of 387 tissue samples from loggerhead sea turtles caught in fish weir (northern Brazil), pair trawl and pelagic longline (southern Brazil), and 123 tissue samples from loggerheads washed ashore in southern Brazil. Sequences (760pb) were classified according to previously described mtDNA haplotypes. Standard genetic diversity indices and genetic differentiation ($F_{st}$ and $\phi_{st}$) were estimated for each foraging aggregation sampled. Natal origins of loggerheads at the study areas were estimated in a Bayesian framework, through many-to-many mixed stock analysis. |
| To verify the occurrence of bottleneck events in loggerhead sea turtles population from SWA | | | ▨ | For this goal of the project, we processed 93 tissue samples of nesting loggerhead females and 21 hatchlings from the main rookeries in Brazil (Sergipe, Bahia, Espírito Santo, and Rio de Janeiro states), and amplified the mtDNA and 10 microsatellite *loci* using two fluorescent dyes (FAM and HEX). Pairwise genetic distance ($F_{st}$ and $D_{st}$) and geographic distance among nesting grounds were calculated. Population structure was also verified through a Bayesian clustering algorithm. Signal of recent genetic bottleneck was tested using three analytical methods: the frequency of rare |

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|---|---|---|---|---|
| | | | ▨ | alleles (L-shaped graphic), the M-ratio, and the Wilcoxon sign-rank test based on a two-phase mutation model. |
| To verify whether natal origins affects growth and trophic ecology of loggerhead sea turtles | ▨ | | | This objective was not achieved because genetic data revealed that the foraging aggregation in southern Brazilian coast, where data on growth and trophic ecology was available from, is composed mainly of a single population, originating from Brazilian rookeries. Also, the low number of early juveniles in the samples did not allow us to fit growth models (see details below in section 2). |

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

The main difficulty that arose was to test the hypothesis that turtles of different natal origins display distinct growth dynamics and feeding behaviour. Although a large sampling of stranded loggerhead sea turtles has been carried out in southern Brazil (n=123), the genetic analyses indicated that 94.1% of loggerheads are from Brazilian population. The remaining specimens showed haplotypes from North Atlantic/Mediterranean Sea (n=2) and Pacific (n=2) rookeries, and hybrids (n=3) with olive ridley (*Lepidochelys olivacea*), but not in a satisfactory sample number to estimate differences in growth and foraging ecology among loggerhead populations. Furthermore, most loggerheads washed ashore were large juveniles and adults, and few specimens were early juveniles (curved carapace length < 55cm, n= 8). The histological processing of humeri of small juveniles did not provide reliable data for age estimation of the loggerheads sampled, due to absorption of the first growth mark (*annulus*). The *annulus* settles closest to the centre of the humerus and later growth marks are deposited sequentially throughout the outer circumference. The age for turtles retaining the *annulus* is defined as the number of growth layers observed in bone sections. However, due to body growth, there is bone resorption in the centre of humerus, leading to the disappearance of the *annulus* and earlier lines, denoted as "lost LAGs". To estimate the age for such turtles, the number of lost LAGs could be estimated using a correction factor, derived from a correlation between the number of growth layers and the corresponding growth layer diameters of individuals that retained the *annulus*. Unfortunately, we were not able to calculate this correction factor with our samples and therefore it was not possible to estimate age and growth of stranded loggerheads. However, the samples from stranded individuals highlighted valuable information about male loggerheads, which are greatly understudied. The SWA is an important habitat for loggerhead sea turtles, with several important nesting and feeding grounds. Most studies at the region have focused on adult females and juveniles, but no information is available for males. Thus, by analysing the skin samples from stranded males and integrating genetic and stable isotope analyses, we obtained the first insights about the origins, movements and foraging ecology of male loggerhead sea turtles in the SWA (see details below in section 3).

The number of samples provided by partner institutions was significantly high and exceeded our expectations. However, an unforeseen difficulty that arose during the project was the quality of DNA samples provided by partners. Samples collected over the last decade, stored in NaCl or collected from turtles in an advanced state of decomposition, showed varying degrees of tissue degradation. For this reason, it was necessary to perform many repetitions and test different extraction methods in order to obtain higher DNA yields. PCR amplifications also had to be performed several times to improve product quality, since genetic analyses requires very concentrated and pure DNA, which caused delays in the original proposed timeframe and increased the cost on sequencing and fragment analyses. About 20% of the total samples processed resulted in no or low-quality genetic data, totalizing 407 mtDNA sequences from foraging grounds, and 101 mtDNA sequence and genotypes from Brazilian rookeries. In any case, although laboratory steps delayed our timeframe, the total number of analysed samples was higher than originally predicted. This was quite positive for the development of the project, as these samples provided a broad dataset and increased the knowledge about loggerheads sea turtles from SWA.

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

Throughout the development of this project, several important results and novel knowledge were obtained about population structure and foraging ecology of loggerhead sea turtles from the Southwestern Atlantic Ocean (SWA). The three most important outcomes are described as follows:

1) *Origin and population structure of loggerheads feeding grounds in SWA.* The SWA represents an important foraging ground of loggerhead sea turtles at different life stages and also holds extensive commercial fisheries. However, the effects of these activities on loggerhead populations remain unclear. In this project, we evaluated the genetic composition of feeding aggregations that overlap with fisheries in northern (fish weir) and southern Brazil (bottom pair trawling, pelagic longline, and stranding). Results indicated significant genetic differentiation between turtles caught in fish weir and pair trawl, as well as between pelagic longline and pair trawl.

A total of 13 loggerhead haplotypes were recorded (Fig. 1). All sampled areas are mostly composed by loggerheads with endemic haplotypes from Brazilian rookeries (CC-A4.1, CC-A4.2, CC-A4.3, CC-A24.1, CC-A25.1): 73.6%, 73.4%, 94.4% and 94.1% of loggerheads caught in fish weir, pelagic longline, pair trawl, and washed ashore, respectively. In northern Brazil, the less frequent haplotypes observed were reported in nesting grounds from the North Atlantic and the Mediterranean Sea (CC-A1.1, CC-A2.1, CC-A3.1, CC-A10.1), and eastern Atlantic (CC-A17.1, Cape Verde rookeries). On the other hand, in southern Brazil we observed haplotypes from rookeries of Pacific (CC-P1.1 and CC-P5) and Indian Oceans (CC-A11.6), as well as haplotype CC-A2.1, which is widespread in the North Atlantic and Mediterranean Sea. In longline bycatch, five orphan haplotypes (i.e., haplotypes that were not previously reported in loggerhead rookeries) were also observed (Fig. 1). Furthermore, two haplotypes characteristic of olive ridley (*Lepidochelys olivacea*) were recorded in specimens from fish weir bycatch (n=6) and from strandings (n=3), indicating hybridisation between species. The estimated contribution of loggerheads rookeries to each sampled foraging aggregation is summarised in Fig. 2. Sea turtles are highly migratory throughout their life cycles and often occupy several habitats under the jurisdiction of multiple countries. Our findings demonstrated that fisheries activities in SWA are impacting loggerhead populations from all ocean basins, but mainly Brazilian populations. The presence of new variants of Indic and Pacific haplotypes in our samples indicate that there are still nesting sites in which genetic composition remains unknown and, consequently, these ocean basins require further studies to ensure adequate population assessment. From a management perspective, our project provides the first broad evaluation of loggerhead foraging grounds in SWA, identifying loggerhead sea turtle populations that form the mixed stocks in northern and southern Brazil. Based on this characterisation, it will be possible to subsidise the creation of fishing restriction areas or the implementation of time-area closures, as well as set up international agreements for effective management of these fisheries, and consequently, promote population stability and preclude population bottleneck events.

*2) Population bottleneck*. The Brazilian nesting population is one of the largest in the world (whose nesting beaches comprises the states of Sergipe, Bahia, Espírito Santo, and Rio de Janeiro), and was recognised as distinct management units within SWA. In Brazil, this species has a long history of threats, marked by ocean pollution, habitat degradation and bycatch in fisheries. One of the possible consequences of population size reduction is the bottleneck effect, when there is an increase in inbreeding and genetic drift, leading to loss of genetic diversity and evolutionary potential. In this project, the genetic diversity and the occurrence of a population bottleneck were evaluated for the first time in Brazilian populations through both mtDNA and nuclear DNA (nDNA), representing both female and male-mediated gene flow. Genetic analysis of mtDNA revealed the presence of one hybrid with olive ridley among nesting female, which was removed from the statistical analyses.

The loggerhead haplotypes identified in this project were similar to previous studies (CC-A4.1, CC-A4.2, CC-A4.3, CC-A24.1), but we report for the first time the occurrence of haplotype CC-A1.3 in Bahia. This haplotype shows high frequency in Cape Verde, in the eastern Atlantic. One *locus* (Cc30) failed to amplify in most sampled specimens and was removed from analysis. Genetic comparisons for both mtDNA and nDNA revealed distinct population structure: whereas mtDNA indicated that Bahia is significantly different from nesting grounds of Sergipe, Espírito Santo and Rio de Janeiro, nDNA showed a high connectivity and no population differentiation among Brazilian rookeries. Microsatellite results also indicated a high frequency of rare alleles among rookeries (Fig. 4). No evidence of a population bottleneck was detected in the Wilcoxon sign-rank test ($P$=0.28) and the graphical method of allelic frequency. The M-ratio showed values at the cut-off point (0.65 ± 0.14) of the analysis. It is assumed that bottleneck population event has occurred when $M < 0.68$ in a data set with $\geq 7$ *loci*. Our results indicated strong philopatry among female loggerheads, but high connectivity among rookeries mediated by males. This male-mediated gene flow could also be mediating the genetic diversity-stability of nesting grounds in the SWA. However, due to the removal of adults and large juveniles to be recruited to the reproductive population by coastal and pelagic fisheries, management efforts are necessary to prevent decline in genetic diversity over the next generations.

3) *Origin and foraging ecology of male loggerheads from southern Brazil.* In marine ecosystems, conducting systematic surveys for collecting baseline data of sea turtle males is generally logistically difficult, since males use the marine environment throughout their entire life cycle and sexual dimorphism is only apparent in the adult life stage, which makes assessing the conservation status and viability of sea turtle populations challenging. Most studies at the SWA have focused on adult females and unsexed juveniles, and little is known about males. In this project, the first insights about origin and foraging ecology of male loggerheads from the SWA were provided by integrating molecular and stable isotope analysis (SIA). Skin samples were obtained from 26 males stranded along the southern coast of Brazil. Samples of potential food sources (benthic and pelagic organisms) were also collected for SIA. mtDNA was sequenced and a Bayesian Mixed Stock Analysis was performed to estimate natal origins of male loggerheads. Bayesian Stable Isotope Mixing Models were fitted to assess the relative contribution of different food sources assimilated by males. Most males exhibited endemic haplotypes from Brazilian rookeries, followed by a low frequency of a haplotype from the North Atlantic and the Mediterranean Sea, as well as olive ridley haplotypes, showing hybridization. SIA showed size-related differences in feeding and habitat use by male loggerheads, with benthic invertebrates dominating the diet of adults, while pelagic prey items dominated the diet of juveniles. Our findings demonstrate the importance of southern Brazil neritic and oceanic habitats for male loggerheads and highlight the value of this area for the maintenance of SWA reproductive management units, which are the main

contributors to these feeding aggregations. This work was recent published in the scientific journal *Marine Biology*.

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

During the development of this project, lectures were given in public schools of the community with the goal of promoting the environmental education of children and teenagers (Fig. 4). In these meetings, the species of sea turtles that occur in the region were described, including their life cycle and ecological features, and the threats that these turtles are currently facing. Moreover, the role of the scientific community in the development of the region was also addressed, as well as the several research projects developed by Laboratório Ecomega, including this project. Indirectly, the goals of our project were shared with fishery communities through the partner institutions, which undertake long-term fishing monitoring and promote environmental education.

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

Yes. The results of our project highlighted that foraging aggregations of loggerhead sea turtles that overlap with pelagic longline hold high haplotype variability and are composed mainly of early juveniles. Our plan is to extend the humeri sampling to longline bycatch in order to attempt to achieve humeri of early juveniles retaining the first growth mark and permit growth and age estimations. This sampling should be conducted in distinct populations of Brazil, especially turtles from the Pacific. One of the partner institutions is already making an effort with fishermen to make this sampling viable.

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

The results of our work have already started to be shared through scientific conferences and meetings. Partial results were shared with scientific community in three Biological Oceanography Meetings that were promoted by Rio Grande Federal University - FURG between 2016 and 2018. Partial results from this work were also presented in the XIII Congresso de Ecologia/III Symposium of Ecology and Evolution that took place in Viçosa city, Minas Gerais state – Brazil - in October 2017 (http://www.ecologia2017.com.br/). Furthermore, partial results were showed at the ASO Marine Turtle Trophic Ecology Workshop, which was held during the ASO Network Meeting on August 2018. 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 the partner NGOs NEMA and Project Tamar. We have published one scientific paper in the high impact scientific journal *Marine*

*Biology* and are currently preparing two additional papers to submit to other high impact journals.

**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 anticipated length of the project was 30 months, but the RSG was used over three 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** |
|---|---|---|---|---|
| Primers for PCR | £306 | £245 | -£61 | Primers acquired were sufficient to carry out the work plan. |
| Taq polymerase for PCR | £367 | £904 | +£537 | Additional Taq polymerase was necessary for additional PCRs. |
| Microtome blade | £248 | £134 | -£114 | |
| Sequencing and genotyping | £1800 | £4379 | +£2579 | The total number of samples provided by partners was significantly higher than was initially planned and additional analyses were required for degraded samples, increasing significantly the costs |
| Stable Isotopes Analysis | £2230 | | -£2230 | Stable isotope analysis of skin samples of male loggerhead turtles and potential food sources were analysed through a partnership with Laboratório EcoMega and the University of New Mexico Center for Stable Isotopes (UNM–CSI), which enabled us to allocate this amount for the sequencing and genotyping. |
| **Total** | £4951 | £5662 | £711 | Approximate exchange rate for period of expenditures: 1£ = 4.04 BRR |

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

The first necessary step is to conclude and submit the two manuscripts that are currently being prepared. Another important step is continuing to share our data with other researchers, conservation projects and managers of protected areas to ensure that effective management plan for the conservation of loggerheads sea turtles will be adopted based on an adequate biological and ecological foundation.

**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?**

The RF was acknowledged in all produced materials. This included all meetings and conferences mentioned above, and in the article published in *Marine Biology*. The RF will be acknowledged in all forthcoming publications involving this project.

**11. Any other comments?**

I would like to express my gratitude to the Rufford Foundation for the financial support. We are sure that the outcomes of our work will be widely application in marine conservation at his Brazilian Exclusive Economic Zone and RSG has been essential to achieve this. I also thank you for understanding that unforeseen setbacks were encountered and we were therefore unable to conclude the project in the original proposed timeframe.

This project constituted my doctoral thesis, which is now being finalised. Looking back, I realise that the project was quite ambitious and that it needed adjustments in sampling design and in the budget to achieve all the objectives completely. However, although the project did not go exactly as predicted, it was possible to achieve almost all of the proposed goals and obtain valuable data for knowledge and conservation of the loggerhead sea turtle populations.

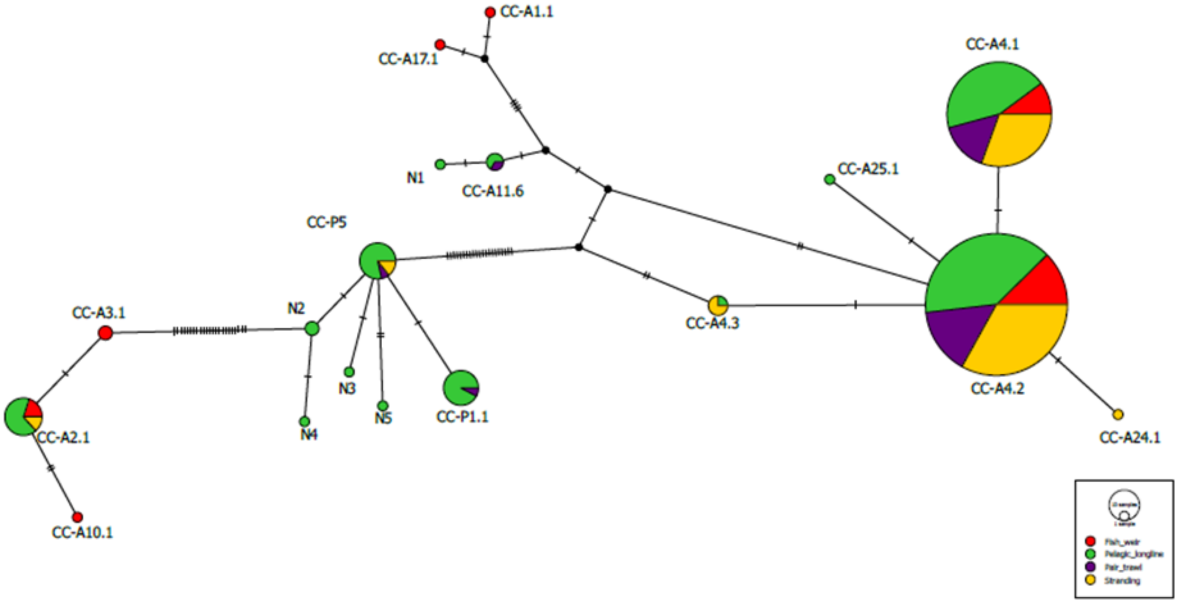

Fig. 1 Network for 760pb of loggerhead haplotypes recorded in southwestern Atlantic Ocean. The circle sizes represent the haplotype frequencies. N1, N2, N3, N4 and N5 represent orphan haplotypes.

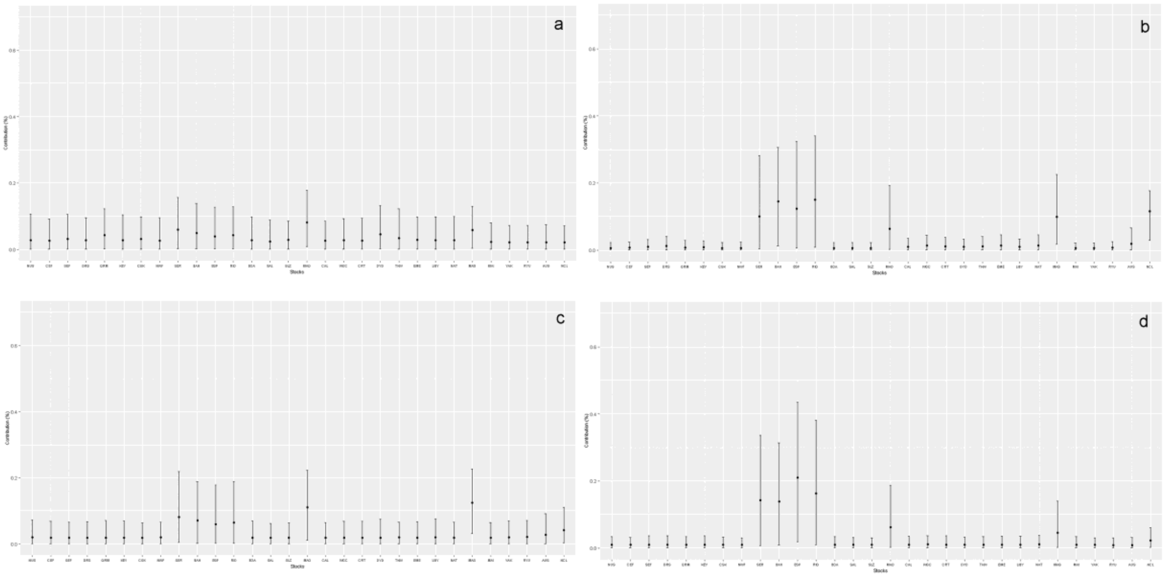

Fig. 2 Contribution proportion and 95% credibility interval of 30 management units to foraging aggregations bycatch in (a) fish weir, (b) pelagic longline, (c) bottom pair trawl, and from (d) stranding. NUS= South Carolina and Georgia states; CEF= northeastern Florida; SEF=southeastern Florida; DRS= south Florida; QRM=Quintana Roo, Mexico; KEY= Keewaydin Island, Florida; CSK= Casey Key, Florida; NWF=northwestern Florid; SER= Sergipe; BAH=Bahia, ESP= Espírito Santo; RIO= Rio de Janeiro; BOA= Boa Vista, Cape Verde; SAL= Sal, Cape Verde; SLZ= Santa Luzia, Cape Verde; CAL= Calabria, Italy; WGR= Greece; CRT= Crete; DYD= Turkey; TKW= western Turkey; EME= Cyprus, Lebanon and Israel; LIB= Libya; NAT= South Africa; MAS= Oman; MAI=Mainland Japan; YAK= Yakushima; RYU= Ryukyu Archipelago; AUS=Australia; NCL= New Caledonia

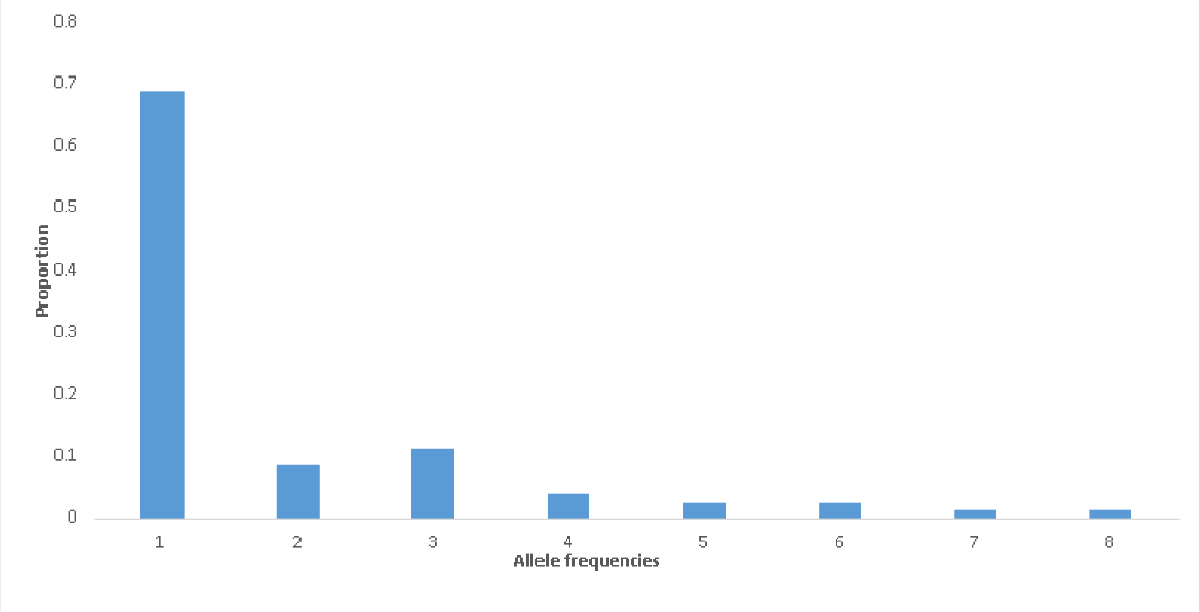

Fig. 3 Allele frequency distributions of loggerheads sea turtles from Brazilian rookeries

Fig. 4 Environmental education of children from the local community