

Project Update: August 2018

The loggerhead sea turtle *Caretta caretta* has a worldwide tropical and subtropical distribution, but global numbers have been greatly reduced over the last decades. In Brazil, this species has a long history of exploitation. Before the 1980s, most of the eggs were collected and many females were slaughtered for food. From this period, with the creation of the Project TAMAR in the main nesting grounds of this species, management plans began to contribute to the recovery of the Brazilian populations of *C. caretta*, with a significant increase in the abundance of nesting females and in the number of nests by season (Marcovaldi & Chaloupka 2007). However, due to the removal of adults and large juveniles to be recruited to the reproductive population by coastal and pelagic fisheries, it is possible that the number of nesting females will decline in the future (Peckham et al. 2011).

One of the 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 (Frankham et al. 1999). Based on the assumption that populations that have recently undergone population bottleneck events have rare allele deficiencies, it is possible to detect events of this type through tests that consider the allelic frequency distribution and the analysis of excess heterozygosity in relation to the total number of alleles (Cornuet & Luikart 1996). In a recent study, nuclear DNA analysis revealed a clear bottleneck event in six of the 13 olive turtle populations (*Lepidochelys olivacea*) from Mexico, revealing a pattern consistent with the demographic imbalance caused by decades of intense commercial exploitation (Rodríguez-Zárate et al. 2013).

In Brazil, available data on population abundance of loggerhead sea turtle preclude the assessment of occurrence of significant decline events of populations. However, along the Brazilian coast there is a high frequency of interspecific hybrids of sea turtles, with nuclear DNA data indicating that this phenomenon is recent (~ two generations or 40 years) and may represent a reproductive strategy due to the lack of partners of the same species (Vilaça et al. 2013). Although the hybridisation process is considered natural in the evolutionary history of organisms, the abrupt decline in population size is one of the possible causes for the emergence of interspecific hybrids (Allendorf et al. 2001).

In this context, knowing the genetic diversity and population structure of loggerhead sea turtles and identifying the occurrence of population bottleneck events is fundamental to establish management practices aimed at restoring metapopulation connectivity and to minimize the additional loss of genetic variability caused by human activities such as fisheries.

Project progress

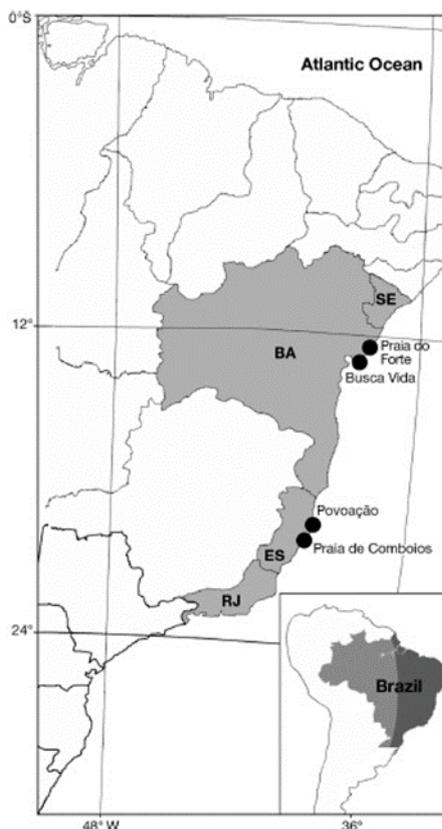
To verify the occurrence of population bottleneck events, we used nuclear markers (microsatellites) previously described for sea turtles (Carreras et al. 2007, Monzón-Argüello et al. 2008). For this analysis, we processed 94 tissues samples of loggerhead nesting females from the main rookeries in Brazil (Figure 1): Sergipe (n=13), Bahia (n=24), Espírito Santo (n=26), and Rio de Janeiro (n=30). The curve carapace length (CCL) of females ranged from 83 to 117 cm (99 ± 0.07 cm). We also processed tissues of 21 loggerhead hatchlings that died inside the nests after hatched from eggs in Praia do

Forte, Bahia. (Fig. 2). The straight carapace length of hatchlings ranged from 3.9 to 4.5 cm (4.1 ± 0.2 cm). All samples from Brazilian rookeries were collected in nesting seasons from 2004 to 2018 and were provided of our partner, the NGO Project TAMAR. The genomic DNA was extracted with PureLink® Genomic DNA Kit. After adjustment of the PCR cycles, 10 microsatellite *loci* were amplified using an Applied Biosystems Veriti 96-well Thermocycler, and are being genotyped (Fig. 3). Additionally, to identify the presence of hybrids in our set of samples, we amplified the mtDNA D-Loop with primers LCM15382/H950 (Abreu-Grobois et al. 2006). The obtained PCR products are currently being purified. After this phase, we will send samples for sequencing in both directions. Haplotypes will be classified according to previously described haplotypes recorded in the Archie Carr Center for Sea Turtle Research database (<http://accstr.ufl.edu/>) and GenBank (<http://ncbi.nlm.nih.gov>).

Closing remarks

Our project has completed 30 months of execution, but there has been a delay in our schedule and we have not yet finalised our activities. Part of the samples destined to amplification of microsatellites were collected in the last reproductive season of loggerheads and could only be sent to our laboratory only in April 2018, which caused changes in our work plan. In September 2018, we will finish the laboratory activities related to the amplification and genotyping of the microsatellites and we will start the analysis of the allele matrix obtained.

In addition, one of the partner institutions of our project has requested the inclusion of more samples of loggerhead sea turtles caught in longline fisheries over the last year and we think it would be appropriate to accept the request in order to improve our assessment of the genetic diversity of populations that are interacting with this fishery. With this inclusion, however, the schedule for data analysis also needed to be modified.



Despite these changes in our work plan, we have already analysed the molecular and stable isotope data of male loggerheads and the scientific paper is already being written. Furthermore, these results were presented at the ASO Marine Turtle Trophic Ecology Workshop, which was held during the ASO Network Meeting on August 16th 2018, in Rio Janeiro, Brazil, by Dr^a Danielle da Silveira Monteiro, a member of our work team. The ASO Network is composed of researchers from Brazil, Uruguay and Argentina who hold annual meetings to discuss methodologies for sea turtle research and conservation strategies with the aim of protecting sea turtles occurring in the Western South Atlantic.

Fig. 1. Location of some of the major loggerhead sea turtle nesting beaches in Brazil. SE = Sergipe, BA = Bahia, ES = Espírito Santo, RJ = Rio de Janeiro. Image source: Marcovaldi & Chaloupka 2007.



Fig. 2. Hatchlings from Bahia collected on the nest season of 2017/2018.

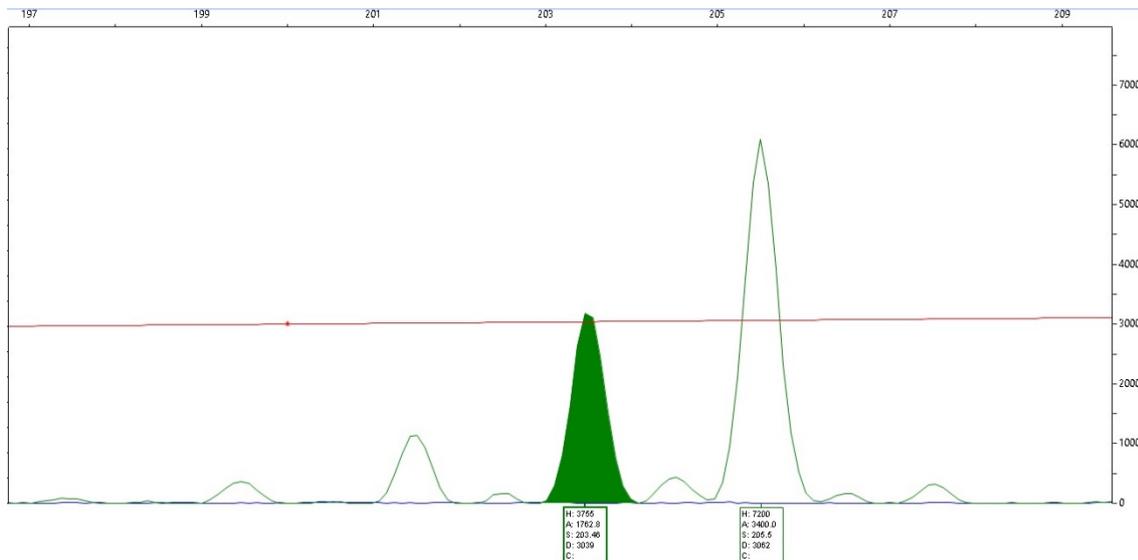


Fig. 3. Alleles peak of one locus genotyped of loggerhead nesting female from Bahia.

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