

## **Project Update: December 2016**

### **Background**

The Southwestern Atlantic Ocean (SWA) represents an important foraging and development ground of loggerhead sea turtles at different life stages (Bugoni et al. 2003), and also holds extensive commercial fisheries (Kotas et al. 2004, Fiedler et al. 2012). A significant overlap between fisheries and sea turtles distribution probably explains the high fishing-related mortality and the decline of several loggerhead turtle populations (Marcovaldi & Chaloupka 2007). However, the effects of these activities on the demographic and genetic structure of loggerhead populations throughout generations remain unclear. Foraging aggregations using both the oceanic and the neritic developmental habitats are mixed stocks composed of individuals from widespread nesting populations (Jensen et al. 2013). Mitochondrial DNA (mtDNA) has been used extensively to investigate the demographic composition of sea turtles' foraging grounds. Previous mtDNA studies revealed the existence of several demographically independent loggerhead turtle stocks composing foraging grounds around ocean basins (Bowen et al. 1995, Reis et al. 2010, Shamblin et al. 2014). In this report, we present preliminary results on the natal origin of 50 loggerhead sea turtles incidentally caught in fisheries on Brazilian feeding grounds, from both oceanic and neritic environments.

### **Partial results**

We had established partnership with Núcleo de Educação e Monitoramento Ambiental (NEMA) and with Project Tamar-ICMBio, which provides tissue samples for DNA analysis from loggerheads caught in fishery activities. To date, from the 378 samples collected, 265 were processed and 132 were sequenced. DNA was extracted with PureLink™ Genomic DNA Kit. The mtDNA D-Loop was amplified through Polymerase Chain Reactions (PCR) with primers LCM15382/H950 and Platinum® Taq DNA Polymerase, using an Applied Biosystems Veriti 96-well Thermocycler under the following conditions: denaturation of 5' at 94°C; 36 cycles of 30'' at 94°C, 30'' at 50°C, 1' at 72°C; final extension of 10' at 72°C. The obtained PCR products were purified with Quick Gek Extraction & Purification Combo Kit PureLink™. Samples were sequenced in forward direction through capillary electrophoresis.

A total of 50 loggerhead sea turtles sequences were analysed. The curved carapace length (CCL) of individuals ranged from 37.5 to 100 cm (mean =  $63.4 \pm 11.8$  cm). Both the commonly used 380pb mtDNA fragment and a longer 800pb fragment from 50 loggerhead individuals were aligned and cropped using BioEdit Sequence Alignment Editor Copyright© (Hall 1999), and classified according to GenBank R○ and the Archie Carr Center for Sea Turtle Research (ACCSTR) website (<http://accstr.ufl.edu/accstr-resources/cclonmtdna.pdf>).

Seven distinct haplotypes were found among the loggerhead turtles sampled from the foraging aggregation: CC-A2.1(2%, n=1), CC-A3.1(2%, n=1), CC-A4.1 (16%, n=8), CC-A4.2 (32%, n=16), CC- CC-A11.6 (6%, n=3) CP1.1 (4%, n=2), and one new haplotype (4%, n=2). It was not possible to estimate the origin of 15 individuals through the forward sequence. In this case, the reverse will be sequenced in order to provide

complementary information. CC-A2.1 is the most geographically widespread haplotype and is present in all of the western Atlantic, eastern Atlantic, and Mediterranean rookeries, except for those in Brazil (Garofalo et al 2009, Reis et al. 2010, Yilmaz et al. 2011). CC-A3.1 has been identified in NW Atlantic rookeries and in Mediterranean nesting sites of Turkey, Lebanon, and Lybya (LaCasella et al. 2013). The haplotypes CC-A4.1 and CC-A4.2 are variants of CC-A4, which is the ancestral and exclusive haplotype of the Brazilian populations (Reis et al. 2010, Shamblin et al. 2014). The haplotype CC-A11.6 was recognized as an Atlantic lineage that colonized the Indian Ocean, mainly Oman nesting sites (Shamblin et al. 2014). The haplotype Cc-P1.1 was previously assumed to characterize Pacific loggerhead populations nesting in Western Australia and Southern Japan (Bowen et al. 1995, Matsuzawa et al. 2016).

A network of haplotypes was constructed using Median Joining Network (Bandelt et al. 1999), implemented with PopArt software (<http://popart.otago.ac.nz>), to visualise differences between haplotypes already reported for the Atlantic and the Pacific Oceans and the new haplotype. As can be observed in the Figure 1, the new haplotype comes from Pacific populations, and it is possible that this haplotype is a variation of the Cc-P1. Our data supports the high migratory behaviour of sea turtles and that juvenile loggerhead turtles traverse the Pacific Ocean to developmental and foraging grounds in the SWA. However, the bycatch in fisheries raises concern over the future of these migratory populations. Therefore, the results presented here are relevant to the ongoing development of conservation plans.

Of all individuals analysed, two were loggerhead-olive ridley hybrids. These hybrids presented the morphology of pure loggerhead turtles and were identified as such, but their mtDNA haplotype was characteristic of nesting olive ridley of Caribbean rookeries (Lo78920, Duchene et al. 2012). Previous studies have reported the occurrence of hybrid offspring along the Brazilian coast (Proietti et al. 2014). The causes behind the extensive hybridization at Brazilian rookeries are still unclear, but are likely a combination of population declines and uneven population sizes of different species (Vilaça et al. 2013).

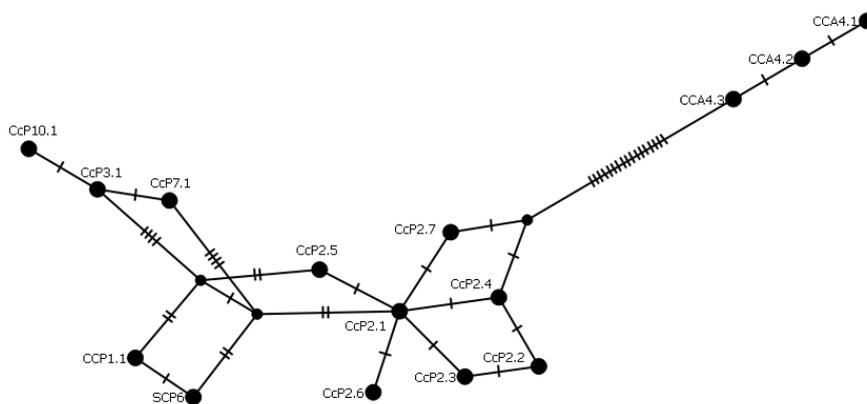


Fig.1. Network between new haplotype found at our study (SCP6) and the haplotypes reported for the Pacific and Atlantic loggerhead rookeries. Dashes along lines indicate differences between haplotypes (1 dash = 1 difference).

## References

- Bandelt H, Forster P, Röhl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Mol Biol Evol* 16: 37–48.
- Bowen BW, Abreu-Grobois FA, Balazs GH, Kamezaki N, Limpus CJ, Ferl RJ. 1995. Trans-Pacific migrations of the loggerhead turtles (*Caretta caretta*) demonstrated with mitochondrial DNA markers. *Proc Natl Acad Sci USA* 92: 3731–3734.
- Bugoni L, Krause MV, Petry. 2003. Diet of sea turtles in southern Brazil. *Chelonian Conserv Biol* 4: 685–688.
- Duchene S, Frey A, Alfaro-Nuñez A, Dutton PH, Thomas P, Gilbert M and others. 2012. Marine turtle mitogenome phylogenetics and evolution. *Mol Phylogenet Evol* 65: 241–250.
- Fiedler FN, Sales G, Giffoni BB, Monteiro-Filho ELA, Secchi ER, Bugoni L. 2012. Drifnet fishery threats sea turtles in the Atlantic Ocean. *Biodivers Conserv* 21: 915–931.
- Garofalo L, Mingozzi T, Micò A, Novelletto A. 2009. Loggerhead turtle (*Caretta caretta*) matriline in the Mediterranean: further evidence of genetic diversity and connectivity. *Mar Biol* 156: 2085–2095.
- Hall, TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp. Ser.* 41:95-98.
- Jensen MP, FitzSimmons NN, Dutton PH. 2013. Molecular genetics of sea turtles. In: Wyneken J, Lohmann KJ, Musick JA (eds) *The biology of sea turtles*, Vol 3. CRC Press, Boca Raton, FL, p 153–154.
- Kotas JE, Santos dos VG, de Azevedo BM, Gallo PC, Barata. 2004. Incidental capture of loggerhead (*Caretta caretta*) and leatherback (*Dermochelys coriacea*) sea turtles by the pelagic longline fishery off southern Brazil. *Fish Bull* 102: 393–399.
- LaCasella EL, Epperly SP, Jensen MP, Stokes L, Dutton PH. 2013. Genetic stock composition of loggerhead turtles *Caretta caretta* bycaught in the pelagic waters of the North Atlantic. *Endang Species Res* 22:73–84.
- Marcovaldi MA & Chaloupka. 2007. Conservation status of loggerhead sea turtle in Brazil: na encouraging outlook. *Endang. Species Res.* 3:133–143.
- Matsuzawa Y, Kamezaki N, Ishihara T, Omuta H, Takeshita K, Goto K, Arata H, Honda K, Kameda Y, Kashima and others. 2016. Fine-scale genetic population structure of loggerhead turtles in the Northwest Pacific. *Endang Species Res* 30:83–93.
- Proietti MC, Reisser J, Marins LF, Rodrigues-Zarate C, Marcovaldi MA, Monteiro DS, Pattiaratchi C, Secchi ER. 2014. Genetic structure and natal origins of immature hawksbill turtles (*Eretmochelys imbricata*) in Brazilian waters. *Plos One* 9: e88746.
- Reis EC, Soares LS, Vargas SM, Santos FR and others. 2010. Genetic composition, population structure and phylogeography of the loggerhead sea turtle: colonization hypothesis for the Brazilian rookeries. *Conserv Genet* 11: 1467–1477.
- Shamblin BM, Bolten AB, Abreu-Grobois FA, Bjorndal KA, Cardona L and others. 2014. Geographic patterns of genetic variation in a broadly distributed marine vertebrate: new insights into loggerhead turtle stock structure from expanded mitochondrial DNA sequences. *PLoS ONE* 9:e85956.
- Vilaça ST, Lara-Ruiz P, Marcovaldi MA, Soares LS, Santos FR. 2013. Population origin and historical demography in hawksbill (*Eretmochelys imbricata*) feeding and nesting aggregates from Brazil. *Journal of Experimental Marine Biology and Ecology* 446:334–344.
- Yilmaz C, Türkozan O, Bardakci F. 2011. Genetic structure of loggerhead turtle (*Caretta caretta*) populations in Turkey. *Biochem Syst Ecol* 39: 266–276.