Project Update: December 2017

Background

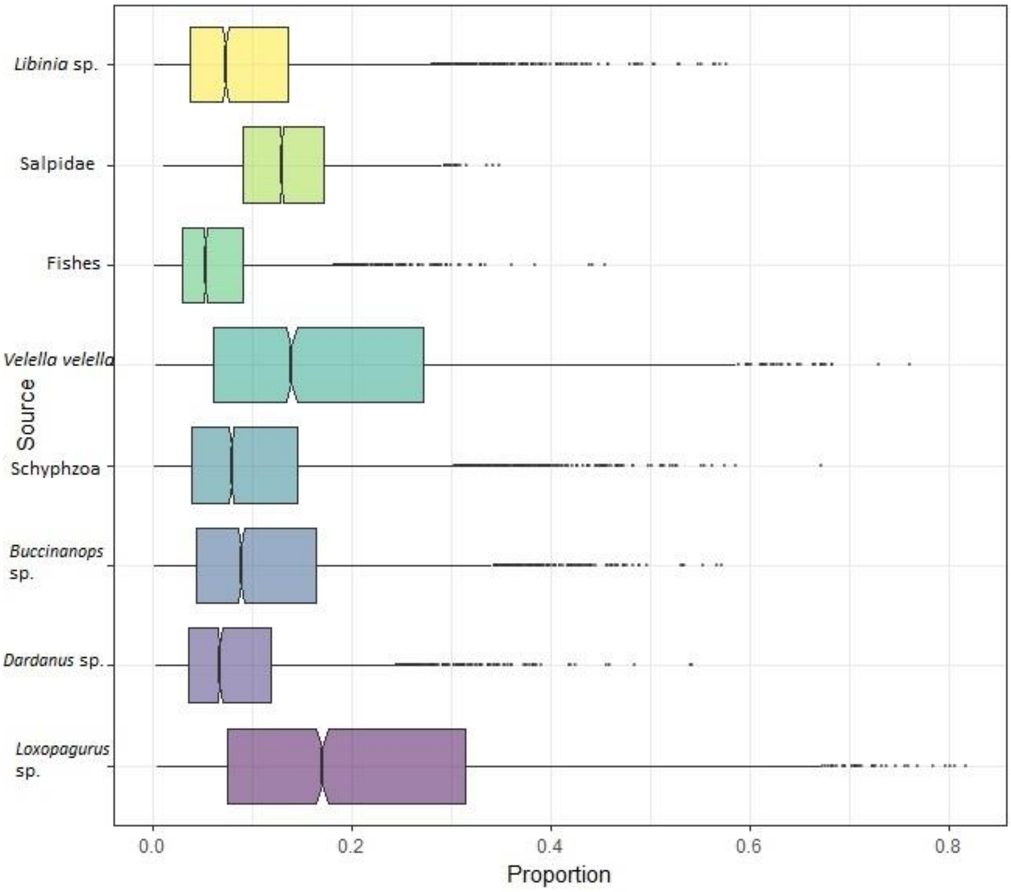
The southwestern Atlantic Ocean (SAO) represents an important foraging ground for loggerhead sea turtles (*Caretta caretta*) at different life stages. However, most studies have focused on adult females and juveniles, and little is known about males. As part of this project, we assess natal origins and feeding ecology of male loggerhead turtles from a southern Brazil foraging ground through genetic and stable isotope analysis (SIA). For both approaches, skin samples were obtained from 21 male loggerheads washed ashore along the southern coast of Rio Grande do Sul state, between February 2014 and February 2017 (Fig. 1). Their curve carapace length ranged from 74.5 to 114 cm (mean = 97.8 ± 9.0 cm). Additionally, samples of potential food sources were collected for SIA. For molecular analysis, DNA was extracted with PureLink™ Genomic DNA Kit. A fragment of the mtDNA control region 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 Gel Extraction & Purification Combo Kit PureLink™. Samples were sequenced in forward and reverse direction through capillary electrophoresis. The sequences obtained were aligned and cropped using BioEdit Sequence Aligment Editor Copyright©, and classified according to GenBank® and the Archie Carr Center for Sea Turtle Research (ACCSTR) website (http://accstr.ufl.edu/accstr-resources/cclonmtdna.pdf). For SIA, a Bayesian Stable Isotope Mixing Model (SIMM) was fitted to stable isotope data to estimate the relative contribution of different food sources assimilated by males. SIMM integrates variability in resource and consumer isotope values, providing a framework from trophic ecology. Food sources are represented by potential food items from neritic and oceanic habitats previously reported for loggerhead sea turtles in SAO.

Partial results

Three haplotypes were identified: CC-A4.1 (25%), CC-A4.2 (70%), and CC-A4.3 (5%). These haplotypes are variants of CC-A4, which is the ancestral and exclusive haplotype of the Brazilian nesting grounds, indicating that males are from Brazilian lineages. $\delta^{13}C$ values ranged from -16.36 to -12.99‰ (-14.31‰ ± 0.97), indicating that these individuals move between the neritic and oceanic habitats. $\delta^{15}N$ values ranged from 10.69 to 18.59‰ (15.78‰ ± 1.69), suggesting that males are feeding at high trophic levels. SIMM demonstrated consistent foraging behaviour among males, with the main food source being *Loxopagurus* sp. (21.0% ± 0.16), followed by the jellyfish *Velella velella* (18% ± 0.14) and salps (13, 3% ± 0.05). These data corroborate studies developed in SAO with loggerheads, which identified high contribution of Brazilian colonies to foraging aggregates and observed a carnivorous diet of benthic organisms at neritic zones and gelatinous prey from oceanic environment.

Partial results from this work were 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/). To the best of our knowledge, this is the first study to infer origin and assess trophic ecology of male loggerhead turtles from SAO. A scientific article with these data set is under preparation with the provisional title "Origin and foraging ecology of male loggerhead turtles revealed by genetic and stable isotope analysis" and will be submitted to *Marine Biology* journal.

Specimen of male loggerhead sea turtle washed ashore in April 2015 during beach monitoring. ©NEMA



Results from SIMM. Colorful boxplots represent the 95% credibility interval, showing estimated prey contributions to male loggerhead sea turtles. Solid black line shows median value, and the vertical lines are the maximum and minimum values.