

Stock Identification of West Indian Manatees *Trichechus manatus* in Northeastern Brazil Through Stable Isotopes.

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Organization

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Project summary:

The West Indian Manatee *Trichechus manatus* is an herbivorous aquatic mammal that inhabits the northern and northeastern Brazil. Because of hunting and habitat degradation, the species is endangered, and its distribution is fragmented and restricted to certain locations with environmental characteristics suitable for its survival. The aim of this project is to identify populations units of *T. manatus* in northeastern Brazilian coast for management purposes. For achieving this, we are going to analyze carbon and nitrogen stable isotope in teeth and bones samples of manatees from different locations, compare the isotopic ratios of these different groups, and relate with the type of vegetation and habitats in which the animals occur. The study will be the first to apply stable isotope analysis on surveys of manatees in Brazil, and will contribute to increase the understanding of trophic ecology and to distinguish discrete populations of *T. manatus*, crucial information in designing management units for conservation purposes of the West Indian Manatee.

Project description

Background:

The West Indian manatee *Trichechus manatus* is a large and herbivorous marine mammal that inhabits the tropical and sub-tropical Atlantic coast. The species is listed in the category "vulnerable" by the IUCN - International Union for Conservation of Nature, and in the Appendice I of the CITES - Convention on International Trade in Endangered Species of Wild Fauna and Flora. In Brazil, is considered endangered in the Action Plan for Marine Mammals.

The species has a coastal distribution in the north and northeastern Brazil, that results in a high overlap with human activities. Historically, manatees have been hunted throughout their range in the country and, together with habitat degradation, are the major threats to its survival. For this reason, the species has a fragmented distribution along the Brazilian coast (Figure 1).



Figure 1 – The fragmented distribution of *T. manatus* along the northeastern coast of Brazil

Since the threats to the manatees and their habitats vary in nature and degree along the species range, it is crucial to identify discrete populations. The identification of discrete population stocks is the first step to evaluate the potential threats to the animals and is essential for the implementation of conservation measures for the species. To establish goals and management strategies in short period of threatened populations, it is necessary to define stocks as "management units" in a less strict sense than the concept of stocks as "significant evolutionarily units", which are defined as historically isolated populations, with low or no genetic exchange. Many studies have proposed stocks using analysis of ecological differences in addition to genotypic information, such as stable isotopic ratios.

Stable isotopes are atoms of the same chemical element that have different atomic masses

and do not decay, such as carbon (^{13}C e ^{12}C) and nitrogen (^{15}N e ^{14}N) isotopes. Because of variations in relative abundance of isotopes, which occur as a result of physical, chemical and biological processes, the proportions between the light and heavy isotopes ($^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$) change with the environments. These ratios are incorporated in the tissues of producers from the assimilation of nutrients of the environment and, through the food chain, incorporated in the tissues of consumers. Thus, stable isotopes can show differences between different regions dominated by different producers.

The use of stable isotopes in studies of marine mammals has grown exponentially, especially with species that live in habitats that make them difficult to observe or it is difficult to directly observe their behavior and feeding habits, such as the manatees. The technique has been used in studies of foraging ecology, habitat use, migration, physiology and paleoecology of marine mammals, and most recently as a tool in the identification of population stocks.

This research is the first to apply the analysis of stable isotopes in studies of Sirenia in Brazil and will expand the knowledge about trophic ecology and habitat use of *T. manatus*, as well as allow identify discrete ecological units, essential information to the development and implementation of strategies for conserving the manatees.

Objectives:

The main goal of the project is to identify management units of the West Indian manatee *Trichechus manatus* along the northeastern Brazilian coast. The approach is based on the conception that animals accumulate in their tissues bioavailable compounds via water and food from their environment. Groups of animals exploiting different habitats may reflect those differences in characteristic element or isotopic compositions of tissues. Thus, they might indicate the existence of “ecological separation”, or management units.

Material and Methods:

The research will be conducted in northeastern Brazil and will be used at least 30 samples of teeth and bones of *T. manatus* carcasses, grouped into five regions where the stranded individuals were found: Alagoas/Pernambuco, Paraíba, Rio Grande do Norte, Ceará and Maranhão states. Triplicate samples will be collected from approximately 20 species of aquatic plants that are part of the diet of manatees, in the same regions where the animals were found stranded.

The samples of teeth and bones will be cleaned with distilled water, dried for 48 hours at

60°C, fractured in small pieces and ground to a fine powder, while the vegetation samples will be cleaned, dried and ground. Sub-samples (1 to 2mg) will be placed in tin capsules and sent to laboratory for determination of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopic ratios, through IRMS - Isotope Ratio Mass Spectrometer. The isotopic ratio is obtained by using the notation δ (expressed in ‰):

$$\delta = X [(R_{\text{sample}} / R_{\text{standard}}) - 1] * 1000,$$

where X is ^{13}C or ^{15}N and R_{sample} and R_{standard} are $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ from sample and reference, respectively.

The values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of teeth and bones will be compared and, if there are no differences, aggregated to determine the isotopic ratios of the five groups of manatees. To determine the contribution of the plants in the diet of *T. manatus*, the isotopic ratios of the sources will be compared with the isotopic ratios of the animals. For this, will be used mixing model, which combines mass balance to isotope sources balance, and the computer program Isosource.

Differences in isotopic ratios of C and N between the groups of manatees from the five regions will be assessed through analysis of one-way ANOVA, followed by Tukey's post hoc test to determine differences between the groups. T-test will be used to compare the values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ between males and females and between adults and sub-adults in the areas. If there are differences between the sexes or between age classes, these factors will be used in the ANOVA above.

Manatees continuously replace their teeth throughout their life, new molars are formed at the back mandible and maxilla, push the other teeth forward and the old ones fall on the front. This substitution could reflect a change in the food history of an individual over time, so, for five individuals, will be determined the values of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of teeth from different positions and bone, and compared to evaluate possibly differences.

Other people/organizations involved in this project

My supervisor, Eduardo Secchi, has a strong background in the ecology and conservation of marine mammals. Silvina Botta has a background in the biology of marine mammals and in the use

of stable isotopes analysis. Ines Serrano and Fábía Luna work at the Centro Nacional de Pesquisa e Conservação de Mamíferos Aquáticos do Instituto Chico Mendes de Conservação da Biodiversidade (CMA/ICMBio), an organization which works with the conservation of the manatees at Brazil. Ana Carolina Meirelles works at Associação de Pesquisa e Preservação de Ecossistemas Aquáticos (Aquasis), an organization which works with the conservation of marine mammals at Ceará state, northeastern Brazil.