Report

Project: Stock Identification of West Indian Manatees *Trichechus manatus* in North-eastern Brazil Through Stable Isotopes

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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 and to study the feeding ecology of *T. manatus* in north-eastern Brazilian coast for conservation purposes. For achieving this, we shall analyze carbon and nitrogen stable isotope in teeth and bones samples of manatees and in the most important plant species that are part of their diet in different locations along their distribution in Brazil. Because the stable isotope values found in the animals and their diet reflect the concentration of these elements in the environment, it is possible to identify ecological groups that present strong site fidelity. In such case, conservation plans that include habitat protection and restoration can be elaborated in a local basis. 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.

Background

The West Indian manatee *Trichechus manatus* (Figure 1) is a large and herbivorous marine mammal that inhabits the tropical and sub-tropical Atlantic coast from Alagoas State (10ºS), Brazil, to Virginia (37ºN), United States. The species is listed as "vulnerable" in the Red Book of the IUCN - International Union for Conservation of Nature, and in the Appendix I of the CITES - Convention on International Trade of Endangered Species of Wild Fauna and Flora. In Brazil, it is considered endangered in the Action Plan for Marine Mammals.

Figure 1 – The West Indian manatee *Trichechus manatus.*

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, represented the major threats to their survival. For these reasons, the species has a fragmented distribution along the Brazilian coast (Figure 2).

Figure 2 – Sightings and strandings of manatees along the Brazilian coast (reproduced from the “Livro Vermelho da Fauna Brasileira Ameaçada de Extinção, Ministério do Meio Ambiente, 2008” - Red Book of the Endangered Brazilian Fauna, Environment Ministry, 2008)

Since the threats to the manatees and their habitats vary in nature and degree along the species range, it is crucial to identify population stocks, which is the first step to evaluate the potential threats to the animals and essential for the implementation of conservation measures for the species on a local basis. Many studies have proposed stock's identification 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 on time, 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 heavy and light isotopes ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) change with the environments. These ratios are incorporated in the tissues of producers (plants or algae) from the assimilation of nutrients of the environment, and, through the food chain, incorporated in the tissues of consumers (in this case, the manatee’s teeth). Since the different habitats used by the marine manatee in Brazil are dominated by different producers, the stable

isotopes measured in their teeth can indicate the food they consumed and the area they inhabited most of the time. It is, therefore, an interesting approach to understand the manatees feeding ecology and habitat use patterns.

The use of stable isotopes in studies on marine mammals ecology has grown exponentially, especially with species for which direct observation of their behavior and feeding habits is difficult, such the manatees. This research is the first to apply stable isotopes analysis to understand the feeding ecology of the marine manatee in Brazil. Hopefully, it will expand the knowledge about trophic ecology and habitat use patterns of this species,. Moreover, it might allow us to identify discrete ecological units, which are essential for the development and implementation of strategies for conserving the manatees.

Objectives

The main goals of the project are to identify management units and to study the feeding ecology of the West Indian manatee *Trichechus manatus* along the northeastern Brazilian coast.

Samples Collection

Teeth and bones samples were obtained from manatee' skulls (Figure 3) deposited in the National Center for Research and Conservation of Aquatic Mammals (CMA/ICMBio) and the Association for Research and Preservation of Aquatic Ecosystems (Aquasis). Samples were assigned to geographic regions based on their collection site: Maranhão, Piauí, Ceará, Paraíba and Alagoas states in the northeastern Brazilian coast.

Figure 3 – A skull of an adult West Indian manatee.

Samples of aquatic plants were collected from most important bays or estuaries occupied by the manatees in the same five regions: Humberto de Campos (Maranhão state), Cajueiro da Praia (Piauí state), Icapuí (Ceará state), Barra do Mamanguape (Paraíba state) and Porto de Pedras (Alagoas state) (Figure 4).

Figure 4 – Sites of collection of plants at northeastern Brazilian coast (© 2011 Google Inc.).

Figure 5 – A subadult male manatee found dead during the field work at Icapuí, Ceará State.

These plants are part of the diet of manatees and belong to distinct groups of aquatic plants: seagrasses (e.g. *Halodule wrightii*), mangroves (e.g. *Rizophora mangle,* Figure 6), seaweeds (e.g. *Cryptonemia crenulata*), and marshgrasses (e.g. *Spartina alterniflora,* Figure 7).

Figure 6 – The red mangrove *Rizophora mangle* at the estuary of Mamanguape River, Paraíba State. The manatees feed upon the leaves of this plant.

Figure 7 – Collection of marshgrass *Spartina alterniflora* at Humberto de Campos, Maranhão State.

Samples preparation

After the sample collection in the field, teeth and bones of the animals as well as the plants were processed at the Turtle and Marine Mammals Laboratory at the Federal University of Rio Grande – FURG.

Teeth and bones were cleaned of outer soft tissue with a carbide burr attached to a drill, cutted with a diamond-embedded blade (Figure 8) and powdered with a small drill bit. The powders were dried for 48 hours in a 60°C oven, acidified with 30% hydrochloric acid for 12 hours and dried again for 1 hour in a 60ºC oven.

Figure 8 – Cutting a tooth of a manatee in a diamond-embedded blade.

The samples of the vegetation were cleaned with fresh water and distilled water, dried for 48 hours in a 60ºC oven and grounded to a fine powder with a mortar and pestle (Figure 9).

Figure 9 – Grounding leaves of a white mangrove *Laguncularia racemosa.*

Isotopic Analysis

Sub-samples of teeth and bones (1.0 mg) and plants (2.5 mg) will be placed in tin capsules and sent to the Analytical Chemistry Laboratory, Institute of Ecology at the University of Georgia, USA, for determination of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopic ratios, through IRMS - Isotope Ratio Mass Spectrometer.

The values of $\delta^{13}C$ and $\delta^{15}N$ of teeth and bones will be used to determine the isotopic ratios of the five groups of manatees. To determine the contribution of the groups of plants in the diet of *T. manatus*, the isotopic ratios of the vegetation will be compared with the isotopic ratios of the animals.

A second Progress Report will be sent as soon as the stable isotopic analysis are finished and a preliminary interpretation of the results is performed.