

Sex determination and sex ratio of Guiana dolphins (*Sotalia guianensis*) incidentally caught in fishing nets along the Northern Brazilian coast

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ABSTRACT

The main objective of this study is to investigate the sex ratio of *Sotalia guianensis* incidentally caught over nine years of monitoring in the coastal zone of the Amazon Forest, precisely in the state of Pará, Brazil, using specific molecular markers ZFX and ZFY. This species is locally threatened by incidental catch and illegal sale of its eyes, teeth and genitals which are utilized as props. In this study it was possible to realize that the sex ratio for Marajó Bay and Salgado Paraense regions are within the expected value of 1:1. This study can help to characterize the population dynamics of this species, since there is a lack of such information, thus making it difficult to determine their current situation and the implementation of practices for their management and conservation.

DESCRIPTION

Introduction

A very large number of cetaceans dies every year, not only because of accidental catch around the world, but also by hunting in some areas, not to mention other well-known threats such as habitat degradation, pollution of aquatic environments and the increased boat traffic (ICMBIO, 2010).

The estuarine dolphin (*Sotalia guianensis*) is the most representative small cetacean of the marine environment of the North coast of Brazil. It is continuously distributed from Honduras, Central America (EDWARDS & SCHNELLS, 2001) to the North Bay in Santa Catarina, in southern region of Brazil (SIMÕES-LOPES, 1988). Due to their preference for coastal habits, they are accidentally caught in fishing nets throughout its range in the northern region of Brazil (EMIN- LIMA et al., 2010).

Most of these carcasses are released back into the water at the fishing site (LODI & CAPISTRANO, 1990). In some cases they have their eyes, teeth and genitals gouged out, which are usually utilized as amulets or handicrafts (SICILIANO, et al., 2005). Thus, most of the

specimens that beach along the coast are already in advanced stages of decomposition, and therefore it's not possible to determine morphologically the sex of the animals.

In these cases, the sex determination can be obtained by molecular markers, like the introns ZFX ZFY located on chromosome X and Y mammals, these introns have been tested in sexual determination of cetaceans (PAIGE et al., 1987). The ZFX is present on the X chromosome and the ZFY on chromosomes X and Y. This method has been successfully used for sex determination of marine mammals by various authors (PALSBOLL et al., 1992; BÉRUBE & PALSBOLL, 1996; SHAW et al., 2003; JAYASANKARET et al., 2008; OLIVEIRA et al., 2009).

The Northern coast of Brazil belongs to coastal zone of the Amazonian biome and it is an almost unknown border on the population dynamics of aquatic mammal groups (SICILIANO et al., 2005). Considering this issue, the sexual determination provides the knowledge of the sex ratio of a species, and then it is possible to establish the disposition of its population structure.

Objective and Methodology

Principal

To determine the sex and sex ratio of Guiana dolphins (*Sotalia guianensis*) accidentally caught in fishing nets along the Northern Brazilian coast, precisely in the coastal zone of the state of Pará, which is in the Amazon Forest.

Specifics

To determine the sex of *Sotalia guianensis* stranded, by molecular markers ZFY and ZFX;

To correlate the sex ratio and the age range with the incidental capture of *Sotalia guianensis*

Study area

Samples of *Sotalia guianensis* tissues were collected through beaches monitoring and service to strandings around the east coast of the Marajó Bay, comprising the coast of the municipalities of Salvaterra and Soure. This portion of the island is part of the large estuary formed by the discharges of large rivers such as the Pará River, Tocantins and some of their smaller tributaries such as Moju, Acará and Guamá rivers. Besides, it has some influence by the Amazon River through the Strait of Breves (GOULDING et al., 2003); and around the salty Pará region, covering the coast of the municipalities of Curuçá, Marapanim and Maracanã,

which are located in the middle region of the northeast Pará state. In this region the ocean waters merge with the continental waters.

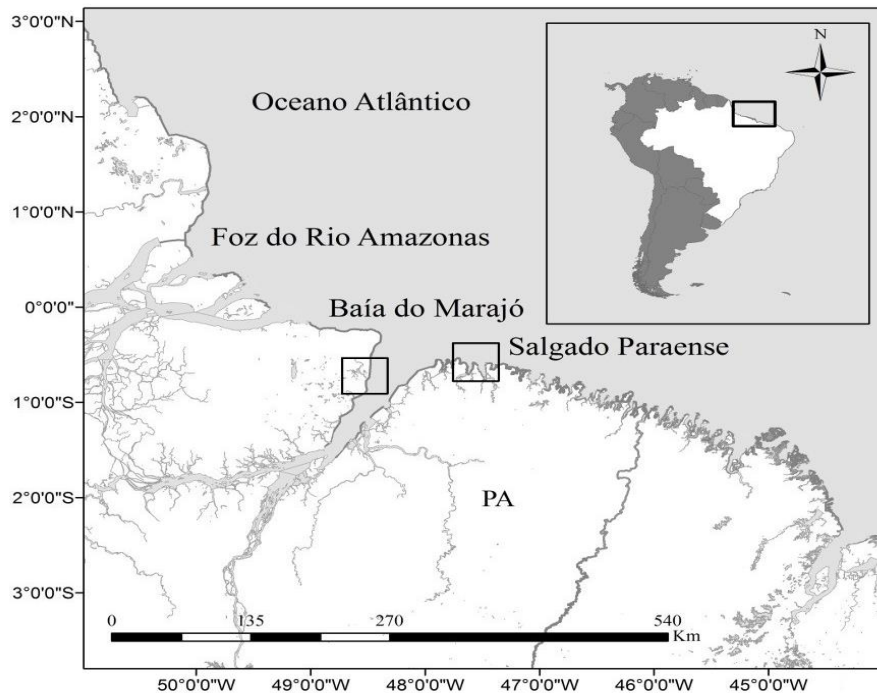


Figura 1: Geographical localization of the monitored areas in which the *Sotalia guianensis* tissues were collected, in Marajó Bay and Salgado Paraense. Source: adapted from VIEIRA, 2009

Collection and Storage of Samples

The samples were collected through beach monitoring, which is done every month according to the tidal conditions of each one of the study areas. In the field, as traces or almost intact animals were found, the material were quantified and specified according to the information required in the field notebook (name of the species, number of record, collection site, geographic coordinates, date of collection, collector, size, type and amount of material found, among others). When the animal was found intact, the default biometrics for Cetaceans were performed, also with the photographic record along with their identification. For each individual in moderate decomposition stage, or still with biological material like muscle and skin samples, the samples were collected with the aid of sterile scalpel blade, and after collection, the samples were stored in the type Eppendorf tubes 1.5 ml and preserved in 98.2% absolute alcohol and storage in freezer (-20 ° C).

All specimens collected was properly identified according to the number of record and the criterion adopted by the Amazon Aquatic Mammals Group (GEMAM). Laboratory

procedures were performed in the Laboratory of Genetics and Conservation Group (GGC-UEMA).

Sex determination

DNA extraction

For DNA extraction from *Sotalia guianensis* some tissue samples were taken using extraction kit (Thermo fisher) according to the manufacturer's instructions. But for the excessively dried samples a special preparation according to a protocol proposed by (MORAES – BARROS & MORGANTE, 2007) was utilized to clean the residues and soften the samples so that they could be submitted to the extraction protocol of (MEDRANO *et al.*,1990). Subsequently, the DNA sample was evaluated in 1% agarose gel, stained with ethidium bromide (1.5 mg / ml) per run in electrophoresis at 100 v for 40 min. For visualization of DNA bands the gel was submitted for viewing on a transilluminator.

Amplification of molecular markers ZFX and ZFY.

The sex determination of the specimens was carried out by PCR amplification (Polymerase Chain Reaction) using molecular markers ZFX and ZFY that are specific DNA sequences located in the X and Y chromosomes of mammals, respectively (PAGE *et al.*, 1987).

The PCR amplification reactions were carried out in a thermocycler prepared in 200 ul microtubes and dimensioned to reach a final volume of 25 uL containing the following reagents: Master Mix (Ludwig Biotec) DNA, ultrapure water and primers ZFY1204 and ZFY0097 (PALSBOLL *et al.*, 1992). The conditions of the PCR cycles are those described by Palsboll *et al.* (1992).

After the PCR, the amplifications were digested by restriction enzyme Taq1 (Ludwig Biotec) for 1 hour in 65° C, the final volume of this reaction is 10 Ul, contain 0,2 taq 1, 1 tampão, 2 DNA and 6,8 ultrapure water. After digestion procedure, amplifications were evaluated in 1.0% agarose gel electrophoresis running at 100 V for 60 or 40 minutes together with molecular weight marker ladder of 100 base pairs (Ludwig Biotec) to estimate the size bands.

The identification of the sex was made through the number of bands that displayed by the transluminator and/or photo documenter. The female ones showed only one band, while males showed two bands. Specimens tissue samples with sex identified, both male and female,

was used a positive control. These samples have passed through all the procedures of those who have unknown gender.

Sex ratio

The sex ratio was performed using the Chi-Square statistical test through BioEstat 5.3 software to see if the observed result has a significant deviation from the expected result in the ratio of 1:1.

RESULTS AND DISCUSSION

It was possible to do a reliable sexing of 70 samples through the established molecular techniques, this number was reduced due to the difficulties encountered in the samples extraction and amplification, as some of these were not properly stored and mainly because most of these animals were classified as IV in the range of decomposition describe for Geraci & Lounsbury (2005). Apart from that, some samples contained fat, which made it more difficult to extract. In addition to the sexed samples, the ones that could be morphologically identified were added in the analysis (n=35). Totaling 101 samples for the analysis.

The analysis was made through the visualization of bands on agarose gel, which for males is observed two bands and females one (picture 2).



Picture 2: *Sotalia guianensis* samples sexed in the Marajó and Salgado Paraense regions.
Legend: Samples 1,2,3,6 and 7 were females and 4,5 males.

Tabela 1: *Sotalia guianensis* samples sexed in the Marajó and Salgado Paraense regions.

Specie	Origin	Sex	Samples identified morphologically	Samples identified by ZFXZFY	Total
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<i>Sotalia guianensis</i>	Marajó	Male	13	14	27
<i>Sotalia guianensis</i>	Marajó	Female	7	32	39
<i>Sotalia guianensis</i>	Salgado Paraense	Male	9	5	14
<i>Sotalia guianensis</i>	Salgado paraense	Female	6	19	25

The chi-square test was made for the two regions ($\chi^2 \alpha = 0.05$, $df = 1$). For the Marajó's region the result found was $p = 0.3821$. Thus, this region will have 1.4 female to one male who suffered the stranding. On Salgado Paraense region, the result was $p = 0.3033$, and for every 1.7 female on a male stranding or capture accidentally. Thus, it is possible to affirm that the sex ratio for both regions are within the expected value of 1:1.

In wild marine mammals populations the sex ratio is not equal to 1:1 due to countless environmental factors in the region (OLIVEIRA *et al.*, 2009). Thus, the sex ratio in relation to specimens beached also various, it depends on the factors that will lead the stranding or the accidental capture.

There are other papers about stranding ratio between males and females, but those use only morphological identification, which is not the case of this paper (HUBNER *et al.*, 2007; MARCONDES & ROSSI-SANTOS, 2004; MEDEIROS, 2006, MARTINS, 2015).

In some regions, the number of males was larger than the females, which were analyzed based on strandings of the *Sotalia guianensis* in the Rio Grande do Norte coast (MARCONDES & ROSSI-SANTOS, 2004; MEDEIROS, 2006), and Pernambuco coast (HUBNER *et al.*, 2007). But according to Martins (2015), in the Amazon region the number of females beached or bycaught accidentally ($n = 13$) was higher than the males's ($n = 7$), which agrees with the results shown in this work.

Vessels and fishing fitting equipments used in Marapanim and Maracana Bay are similar to those used in Marajó Bay, both are specially used to artisanal fisheries. The gillnets were the most used ones in both areas (QUEIROZ, 2004; ISAAC *et al.*, 2006) and are the most threatening ones to the small cetaceans, including *Sotalia guianensis*.

This type of fishing equipments may be related to the sex of the animals, because males and females have size differences (RAMOS, 2000) and that fact can influence the entrapment of these animals.

In the Amazon region in Marajó Bay, the vegetation is characteristic of mangroves and can also affect in the strandings, because many carcasses are found near the mangrove roots, the area also features lots of bays and estuaries (SOUZA-FILHO, 2005), increasing the animals presence in the region.

Population studies with *S. guianensis* are still scarce, especially in these study areas (EMIN-LIMA,2010) and don't have other published works in these areas using this technique. But it was used in others areas with *S. guianensis* for CUNHA & SOLÉ-CAVA (2007), and in other cetaceans for PALSOLL (1992); PALSOLL & BERUBÉ (1996), ROSEL (2003), OLIVEIRA *et al.* (2009).

The ZFXZFY technique allows to know and expand the data available to define the sexual strandings pattern, offers new insights into *Sotalia* social structure and improves conservation and management decisions (CUNHA & SOLÉ-CAVA, 2007).

CONCLUSION

This technique was efficient for animals sex determining mostly when it wasn't possible to determine it morphologically. The results obtained in both regions through the statistical chi-square test were significant to the expected values.

Thus, through the sex ratio it is possible to know the population specimen organization. And this knowledge was important especially in ecologically important areas and places that haven't been studied, like the areas in this paper.

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