Populations analysis of the Brazilian Sharpnose Shark

*Rhizoprionodon lalandii* (Chondrichthyes: Carcharhinidae) on the São Paulo coast, Southern Brazil: inferences from mt DNA sequences

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Sharks of the genus *Rhizoprionodon* can be considered some of the most important predators along the trophic coastal marine ecosystems and represent an important economic resource for the small-scale fisheries, especially on the Brazilian coastline. In order to analyze the population structure of the shark *Rhizoprionodon lalandii* of São Paulo, Southeastern coast of Brazil, levels of genetic diversity were identified by nucleotide sequence analyses of the mitochondrial DNA control region. The results obtained from this study present moderate values of haplotype diversity and low nucleotide diversity. Although the AMOVA tests ($\Phi_{ST} = 0.08394$, $P < 0.01$) had shown slightly differences among the studied samples, evidence for the occurrence of population structuring was not found, which may be a general feature of sharks living in coastal areas.

**Key words:** D-loop, Haplotypes, Mitochondrial control region, Shark conservation.

**Introduction**

Fishery resources are considerably important for the maintenance of the ecosystems and of significant value as food. Although the exploitation of sharks and rays for eating, medical, and religious purposes has been practiced since the 16th century, a significant increase in its capture has been observed (Stone et al., 1998). Statistical data from FAO (Food and Agriculture Organization of the United Nations) show that the total world capture of sharks and rays corresponded to 823,844 and 828,364 tons in 1999 and 2000, respectively. From this total, Brazil was responsible for 2.25% (18,553 t) in 1999 and 2.23% (18,480 t) in 2000 (FAO, 2002). However, 31,300 tons of sharks and rays were captured in Brazil in the 1980’s decade, a period that represented the golden years of the elasmobranch fishery in the country (Bonfil, 1994). Unfortunately, there is a lack of recent statistical data in Brazil, and an up-to-date prospect of the fishery situation in the country is not available.

The worldwide management of elasmobranch stocks has been impaired by the lack of basic information on their population dynamics. The available data on Brazilian coast sharks are restricted to few species that can be also found on the Red List of Threatened Species of the IUCN (International Union for the Conservation of Nature and Natural Resources) (Camhi et al., 1998).

The sharks of the genus *Rhizoprionodon* have a worldwide range, usually associated with coastal areas, however few studies about these fish were published (Sadowsky, 1967; Lessa, 1986; Kasim, 1991; Grace & Henwood, 1997) and some species of the genus *Rhizoprionodon* are included on the IUCN

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Red List of Threatened Species (www.iucn.org, 2006).

The Brazilian sharpnose shark *Rhizoprionodon lalandii* is a small size species that belongs to the family Carcharhinidae, which occurs in the Atlantic Ocean, from Panama to Argentina (Figueiredo, 1977; Compagno, 1984). Due to its abundance and occurrence on the coastal waters, it plays an important role as a predator in the marine coastal ecosystem, and constitutes an important economic resource for the small-scale fisheries. In Brazil, this species is qualified by the IUCN as vulnerable. Fishing of elasmobranches has systematically increased on the coast of Brazil, this species is qualified by the IUCN as vulnerable. Fishing of elasmobranches has systematically increased on the coast of the State of São Paulo since 1996 (Gadig et al., 2002). According to Motta et al. (2005), *R. lalandii* is the most captured shark species, representing 60% of all sharks caught in this region.

The number of expressive studies about the genetic structure of sharks and rays populations along the Brazilian coast is quite reduced, contrasting with the increasing number of elasmobranches captured, the continued inclusion of new species as endangered or at risk of extinction, and the lack of appropriate knowledge for the sustainable management of these exploited species. Thus, the aim of the present study was to search information that can support the establishment and characterization of the population structure of the Brazilian sharpnose shark, *Rhizoprionodon lalandii*, on the São Paulo coast, Southern Brazil, as well as offer support management strategies for the sustainable exploitation of this species.

**Material and Methods**

**Samples characterization.** Samples of *Rhizoprionodon lalandii* were obtained from local fishermen communities, sampled, and identified and vouchers were kept in the fish collection of Laboratório de Biologia e Genética de Peixes (LBP), Departamento de Morfologia, Instituto de Biociências, UNESP, Botucatu, São Paulo, Brazil. Thirty-five samples (13 neonates, 14 juveniles and 8 adults) were collected in Itanhaém (24°11′01″S, 46°47′18″W) (voucher number: LBP 3001), 22 samples (10 neonates and 12 juveniles) in Praia Grande, (24°00′35″S, 46°24′45″W) (voucher number: LBP 3154), and 37 samples (16 neonates, 11 juveniles and 10 adults) in Ubatuba (23°26′15″S, 45°03′45″W) (voucher number: LBP 3006), all of them in the State of São Paulo, Brazil, between March and December 2005.

**DNA extraction, amplification by PCR, and nucleotide sequencing.** Total genomic DNA was extracted from the branchial tissue, using the phenol/chloroform protocol, according to Sambrook & Russel (2001). The amplification of the D-Loop mitochondrial segment (1300 bp) was achieved by Polymerase chain reaction (PCR). The amplification reaction included 25μl of solution containing 0.8 mM of dNTP, 1.5 mM of MgCl₂, 1x buffer reaction (20 mM Tris-HCl pH 8.4, and 50 mM KCl), 100 ng of primers (F - 5′ CTC CCA AAG CCA AGATTCG - 3′ and R - 5′ - GCC TTAGGAGT GGT TTCT CTT GG - 3′) according to Cao (1998) and 1 unit of enzyme Taq DNA Polymerase (Invitrogen). Each round consisted of 35 PCR cycles. Each cycle consisted of denaturation at 95°C for 1 minute, annealing at 55°C for 30 seconds, and 1-minute extension at 72°C. The amplified segments of DNA were visualized on 1% agarose gel stained with Ethidium Bromide. Purified sequencing reactions were electrophoresed in denaturing polyacrylamide gels on an ABI PRISM 377 DNA sequencer.

**Data Analyses.** Mitochondrial Control region sequences were aligned using the program DAMBE (Xia, 2001) and checked by eye. The nucleotide composition, sequence diversity, number of polymorphic sites, number of transitions and transversions, haplotype diversity, and haplotype number were calculated using ARLEQUIN version 3.01 (Excoffier & Schneider, 2005). Analyses of molecular variance (AMOVA) (Excoffier et al., 1992) were conducted to examine spatial genetic heterogeneity among nurseries for both control region haplotypes, using ARLEQUIN 3.01. Significance of Φ-statistics was determined via nonparametric permutation (Excoffier et al., 1992), with 1000 data permutations.

**Results**

Although the PCR amplification of the D-Loop mtDNA generated a fragment of approximately 1300 bp, the nucleotide sequence was determined for a segment of 514 bp of the most variable region (5′ extremity), which was composed of 32.48% adenine, 37.18% thiamine, 20.48% cytosine, and 9.86% guanine. The prevalence of a higher AT content was evidenced in the control region of the mitochondrial DNA in the species *Rhizoprionodon lalandii*. Twenty one sites were variables, resulting in 16 haplotypes (Table 1) with values of haplotype diversity, 0.8239, and nucleotide diversity, 0.004843 ± 0.002941, calculated according to Nei (1987).

Haplotypes 1, 2, 3, 6, 10, 11, 14, and 16 were found in 83 samples, which involves 88.3% of the analyzed sharks. Regarding these, 34 were sampled on the Ubatuba, 30 on the Itanhaém, and 21 on Praia Grande coast. The other original haplotypes were distributed among the three areas without repetitions (Table 2). Three well-represented haplotypes were noticed within the haplotype network (Fig. 1): H1, H2, and H3. It is worth highlighting that no separation in groups of geographic samples was observed, and that the most representative haplotypes are found in all three localities. However, the analysis of molecular variance showed a value corresponding to a moderate structuring among populations (ΦST = 0.08394, P < 0.01), among populations within groups (ΦSC = 0.05487, P < 0.01), and among groups (ΦCT = 0.03076, P < 0.01).

**Discussion**

Our results suggest moderate values of haplotypes diversity, low nucleotide diversity and a high degree of gene flow among samples of Brazilian sharpnose shark collected on the São Paulo Coast. However, a moderate structuring was observed among populations, among populations within groups, and among groups. These results are similar to those found by Heist et al. (1996) with the species *Rhizoprionodon terranovae* on the U.S.A Atlantic coast and in the western
Rhizoprionodon may have only a weak populational structure in relatively wide coastal areas, as observed in the present study and by Heist et al. (1996). This observation may also be true for other shark species as Carcharhinus plumbeus for which Heist et al. (1995) studying populations from the Gulf of Mexico and Mid-Atlantic Bight also found low levels of genetic variability.

The nucleotide diversity was very low (0.161) with 87 of 95 individuals sharing the common genotype. On the other hand, studies conducted in more wide areas may have only a weak populational structure in relatively wide coastal areas, as observed in the present study and by Heist et al. (1996). This observation may also be true for other shark species as Carcharhinus plumbeus for which Heist et al. (1995) studying populations from the Gulf of Mexico and Mid-Atlantic Bight also found low levels of genetic variability. The nucleotide diversity was very low (0.161) with 87 of 95 individuals sharing the common genotype.

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indicate that other shark species have populations strongly structured, as was observed for Carcharias taurus ($\Phi_{ST} = 0.295; P < 0.001$), showing an insignificant migration between the eastern and western Australia and southern Africa (Stow et al., 2006). A strong structuring (mitochondrial $\Phi_{ST} = 0.350, P < 0.001$; nuclear $\Phi_{ST} = 0.007, P < 0.001$) was also reported for Carcharias limbatus caught between the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea (Kenney et al., 2005) and for the hammerhead shark (Sphyrna lewini) from different ocean population subdivisions (overall $\Phi_{ST} = 0.749, P < 0.0001$ and among oceans $\Phi_{ST} = 0.598, P < 0.0098$). In these cases, genetic discontinuity within oceans ($\Phi_{ST} = 0.519, P < 0.0001$) was primarily associated with oceanic barriers (Duncan et al., 2006).

Therefore, it is possible to conclude that there is a tendency for low population structure or even for an absence of population structure among shark samples from restricted areas and moderate to high population structuring among shark samples from wide areas, even without strong barriers. Further, this hypothesis has to be tested with samples from a wide distribution area sampled in as many intermediate points as possible. However, the identification of a moderate structuring among populations of Rhizoprionodon lalandii from the coast of São Paulo should be carefully appraised, because if this phenomenon is a rule for to be applied to this species, it will be very important to develop conservation politics for each of the units detected.

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