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NOTE

STOCK STRUCTURE OF THE SWORDFISH (*XIPHIAS GLADIUS*) IN THE SOUTHWEST INDIAN OCEAN: A PRELIMINARY STUDY

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The swordfish (*Xiphias gladius* Linnaeus, 1758) is a large oceanic migratory apex predator distributed globally, ranging from tropical to cold waters (Carey and Robinson ,1981; Palko et al., 1981; Nakamura, 1985). This species is taken mainly by targeted longline fisheries throughout the Indian Ocean. Catches in the Indian Ocean increased sharply in the 1990s, peaking in 1998 at around 35,000 t, and decreasing slightly over the last 5 yrs. Despite this decline, the effective fishing effort has continued to increase over this period. This pattern is thought to reflect a decrease in the swordfish biomass, and potentially, overfishing (Report of the Eighth Session of the Scientific Committee of the IOTC). The lack of knowledge about the stock structure and migratory behaviour of swordfish hinders the development of a shared management strategy of this important Indian Ocean marine resource.

Molecular techniques have been successfully developed to study large pelagic fishes stock structure, interchange, and spawning between regions (Baker et al., 1990; Barlett and Davidson, 1991; Durand et al., 2005; Duncan et al., 2006). Recent genetic studies on mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) have revealed at least four distinct stocks of swordfish in the world (Alvarado Bremer et al., 2005) : (1) Mediterranean (Kotoulas et al., 1995; Alvarado Bremer et al., 1996; Rosel and Block, 1996; Chow et al., 1997; Kotoulas et al., 2003; Reeb et al., 2003), (2) North Atlantic, (3) South Atlantic (Alvarado-Bremer et al., 1996; Chow et al., 1997), and (4) Indo-Pacific (Chow et al., 1997; Chow and Takeyama, 2000), with a subtle structure in the Pacific (Reeb et al., 2000). No genetic heterogeneity has been reported between the Indian and the Pacific oceans (Chow et al., 1997; Chow and Takeyama, 2000), but genetic studies on swordfish remain insufficient in the western Indian Ocean to examine the possibility of an intra-ocean stock structure.

Before starting a large scale study on the population structure of the swordfish in the Indian Ocean, we conducted a pilot study in a restricted area (southwest) using both mtDNA and microsatellite loci markers in order to validate protocols and to analyze the relatedness of the sampling locations.

Materials and Methods

A total of 90 samples was collected between February and May 2005 from four different fishing zones of the South West Indian Ocean : Reunion Island (20), southern Madagascar (30), southern Mozambique Channel (20), and Seychelles Archipelago (20). Sampling was carried out after landing for the three first locations, and onboard a fishing vessel for the last. Muscle tissues were taken using a sterilized scalpel, stored in ethanol 90% or 20% Dimethyl Sulfoxide (DMSO) saturated salt solution (Dutton, 1996), and then frozen. DNA was extracted from small amounts of tissue using Chelex (Bio-Rad) following the procedures of FitzSimmons et al. (1997). DNeasy Tissue Kit (Qiagen) was also tested and found successful.

mtDNA.—Segments of the cytochrome b and 12S rRNA genes, and the control region and flanking tRNAs were amplified in order to perform a Restriction Fragment Length Polymorphism (RFLP) using two restriction endonucleases (RsaI and AluI, New England Biolabs). The

primers used were as follows: CB3R-L, 5′–CAT ATT AAA CCC GAA TGA TAT TT–3′ and 12SAR-H, 5′–ATA GTG GGG TAT CTA ATC CCA GTT–3′ (Chow et al., 1997). PCR reaction contained 1× buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.5 μM of each primer, 2 units of Taq DNA polymerase (Eurogentec Red Gold Star), 25 ng of total DNA, in a final volume of 25 μl. Cycling parameters and restriction endonucleases digestions protocols are described in Chow and Inoue (1993). Restriction patterns observed for each endonuclease digestion were alphabetically labelled and the frequencies of composite haplotypes compared among samples.

Microsatellite Loci.—Six microsatellite loci (Xg-55, Xg-56, Xg-66, Xg-75, Xg-144, and Xg-166) described in Reeb et al. (2003) were amplified using the same PCR reaction defined for mtDNA with 0.25 units of Taq DNA polymerase, and using cycling parameters described in FitzSimmons et al. (1997). Amplified fragments were separated on an ABI Prism 3100 genetic analyser. Alleles were scored using a comigrating size standard (Genescan-500, Applied Biosystems, Inc.) and identified using GeneScan 3.7 (Applied Biosystems, Inc.).

Data Analyses.—Data analyses were performed on Arlequin, v. 2.0 (Schneider et al., 2000). Microsatellite data were tested for significant departures from Hardy-Weinberg equilibrium. For both mtDNA and microsatellite loci, differentiation between pairs of populations was assessed with Wright's fixation index F_{st} (1023 replicates ; Wright, 1951). Population genetic structure was tested using analysis of molecular variance (AMOVA, 1023 replicates; Excoffier et al., 1992) among all the samples and also by testing various alternative groupings.

Results

mtDNA.—Eighty-six samples of approximately 1900 base pairs were amplified. Endonuclease digestions resulted in seven restriction patterns in RsaI and four in AluI, yielding a total of 15 haplotypes combining the restriction patterns of the two endonucleases, five of which were most frequent (Table 1). Haplotypic diversity for each population was very high, with a value of 0.852 for the pooled samples, and varied from 0.789 (Seychelles Archipelago) to 0.896 (southern Madagascar; Table 1). Despite appearance of complex haplotype frequencies at each location, AMOVA revealed no genetic structure between the four populations (global F_{st} = 0.01), with nearly all haplotypes variation occuring within locations (98.94%). Alternative groupings were also tested but none was statistically significant. Pairwise comparison of the four locations showed very low values of F_{st} (0.0017–0.02) with non-significant P values ($P > 0.197$; Table 2).

Microsatellite Loci.—Seventy-one samples were amplified for the six microsatellite loci. Total number of alleles per locus was found to be highly variable : very high for loci Xg-75 (32 alleles) and Xg-55 (26 alleles), and much lower for the others (< 16 alleles). The mean number of alleles per locus was 10, ranging from 7.17 in the Seychelles Archipelago sample to 13.5 in the southern Madagascar sample (Table 3). Loci size ranges between sites were variable for all loci, except Xg-66 for which the size range conformed for all the locations (Table 3). The observed genotype number in each population generally conformed well with Hardy-Weinberg equilibrium for four loci, but two loci significantly deviated from expectations for the four locations (Xg-55 and Xg-75, $P < 0.007$; Table 3) showing heterozygote deficit in all locations, and locus Xg-56 for Mozambique Channel. Pairwise comparison of the four locations did not show significant differentiation (F_{st} = [–0.002; 0.0008], $P > 0.14$; Table 4).

Table 1. Swordfish mtDNA. Number of composite haplotypes (% frequencies) and haplotypic diversity within four sample sites in the South West Indian Ocean on the basis of restriction patterns in digestions by RsaI and AluI (the five major haplotypes are in bold).

Haplotype	Seychelles Archipelago	Reunion Island	Southern Madagascar	Southern Mozambique Channel	Total individuals
AA	9(45)	5(28)	6(32)	6(21)	**26**
AB	1(5)	1(6)	1(5)	5(17)	**8**
AC	3(15)	3(17)	0(0)	2(7)	**8**
BA	1(5)	4(22)	5(26)	4(14)	**14**
BB	2(10)	0(0)	4(21)	4(14)	**10**
BC	1(5)	1(6)	0(0)	4(14)	6
BD	1(5)	0(0)	0(0)	0(0)	1
CA	0(0)	0(0)	1(5)	0(0)	1
CC	0(0)	0(0)	1(5)	0(0)	1
DB	1(5)	0(0)	0(0)	1(3)	2
EA	0(0)	0(0)	0(0)	1(3)	1
EC	0(0)	2(11)	0(0)	0(0)	2
FA	0(0)	0(0)	1(5)	0(0)	1
FC	1(5)	1(6)	0(0)	2(7)	4
GC	0(0)	1(6)	0(0)	0(0)	1
Total individuals	20	18	29	19	86
Total haplotypes	9	8	9	7	
Haplotypic diversity	0.789	0.869	0.896	0.817	0.852

Discussion

This preliminary study allowed us to define an extraction protocol, and to adapt amplification and endonuclease digestion methods. No genetic structure could be found among the swordfish sampled at the four locations using either molecular technique. Most variability was found within populations, with only a small proportion of the total variation among samples, suggesting a unique stock of swordfish in the South West Indian Ocean.

Other studies based on mtDNA (Chow et al., 1997; Chow and Takeyama, 2000; Alvarado Bremer et al., 2005) and microsatellite loci (Kotoulas et al., 2003; Reeb et al., 2003) have used sample sizes > 20 individuals from multiple oceans to show significant divergence among swordfish populations in other oceans. Thus, it is possible that the geographical scale and small sizes in the present study may not be large enough to detect differences that are present, and a larger sampling effort is needed

Table 2. Swordfish mtDNA. Pairwise comparisons of the four locations showing F_{st} (below diagonal) and P values (above diagonal).

	Seychelles Archipelago	Reunion Island	Southern Madagascar	Southern Mozambique Channel
Seychelles Archipelago	*	0.37488 ± 0.0086	0.21785 ± 0.0086	0.19702 ± 0.0078
Reunion Island	0.00170	*	0.34612 ± 0.0085	0.29190 ± 0.0073
Southern Madagascar	0.01505	0.00423	*	0.23769 ± 0.0083
Southern Mozambique Channel	0.02071	0.00818	0.01251	*

Table 3. Swordfish microsatellite loci. Allele statistics and sizes for each locus from each sample group.

Locus	*n*	*a*	H_O	H_E	P_{HW}	Size range (bp)
Seychelles Archipelago	9					
Xg-55		8	0.28571	0.96703	0.00000	98–147
Xg-56		13	0.88889	0.95425	0.53615	116–146
Xg-66		8	0.88889	0.86928	0.75421	120–138
Xg-75		9	0.50000	0.95833	0.00109	166–235
Xg-144		3	0.50000	0.61667	1.00000	157–163
Xg-166		2	0.00000	0.64286	0.14278	129–132
Mean		7.17				
Reunion Island	14					
Xg-55		13	0.21429	0.96825	0.00000	91–144
Xg-56		12	0.78571	0.91534	0.40547	116–143
Xg-66		8	0.85714	0.86243	0.96128	120–136
Xg-75		13	0.46154	0.94154	0.00000	166–218
Xg-144		5	0.57143	0.70370	0.42148	157–169
Xg-166		7	0.66667	0.72464	0.45142	120–138
Mean		9.7				
Southern Madagascar	30					
Xg-55		23	0.76667	0.96384	0.00352	87–154
Xg-56		14	0.78571	0.85844	0.69711	116–160
Xg-66		8	0.76667	0.85141	0.35109	120–138
Xg-75		23	0.72414	0.94979	0.00000	160–232
Xg-144		5	0.53333	0.65480	0.29770	154–169
Xg-166		8	0.62069	0.59286	0.91859	120–145
Mean		13.5				
Southern Mozambique Channel	18					
Xg-55		16	0.64706	0.95187	0.00000	91–133
Xg-56		10	0.55556	0.86032	0.00977	116–146
Xg-66		7	0.83333	0.77143	0.83780	120–136
Xg-75		15	0.66667	0.95397	0.00684	172–237
Xg-144		4	0.58824	0.66310	0.24278	154–163
Xg-166		7	0.58824	0.60606	0.34764	120–141
Mean		9.8				

Number of individuals analyzed (*n*), number of alleles (*a*), observed heterozygosity (H_O), expected heterozygosity (H_E), Hardy-Weinberg probability test (P_{HW}), and size range of the loci.

Table 4. Swordfish microsatellite loci. Pairwise comparisons of the four locations showing F_{st} (below diagonal) and P values (above diagonal).

	Seychelles Archipelago	Reunion Island	Southern Madagascar	Southern Mozambique Channel
Seychelles Archipelago	*	0.99902 ± 0.0002	0.99902 ± 0.0002	0.54492 ± 0.0158
Reunion Island	–0.00199	*	0.99902 ± 0.0002	0.49609 ± 0.0147
Southern Madagascar	0.00000	0.00000	*	0.14844 ± 0.0113
Southern Mozambique Channel	0.00082	0.00080	0.00078	*

to confirm these results. Should additional sampling confirm the hypothesis of a unique stock in this region, samples may be pooled for comparisons of the Indian ocean population to more distant populations. The inclusion of other biological (sex and individual size) and environmental (seasonal and oceanographic) parameters will eventually enable us to better understand migratory behavior of the swordfish and provide more precise information for managing swordfish in the Indian Ocean.

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