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Stock structure of the swordfish (Xiphias gladius) in the southwest Indian Ocean: A preliminary study

Claire Jean[1], Jérôme Bourjea[*,1], Emmanuel Jouen[2] and Marc Taquet[1]

[1] Ifremer La Reunion, F-97822 Le Port, Reunion
[2] Ctr Rech & Cooperat Int Rech Agron Dev, CIRAD, Lab Pathol & Genet Mol, F-97410 St Pierre, Reunion

*: Corresponding author : Jerome.Bourjea@ifremer.fr

Abstract:

Stock structure of swordfish (*Xiphias gladius* Linnaeus, 1758) from four areas of the South West Indian Ocean was conducted using Restriction Fragment Length Polymorphism (RFLP) on mtDNA control region and six amplified microsatellite loci. No significant differences were found among the four locations using either molecular technique. Most variability was found within populations, with only a small proportion of variation among samples, suggesting a unique stock of swordfish in the South West Indian Ocean. The sample size of this study is too small to reveal significant structure among the sampling locations. These results are baseline for a future larger scale project in the whole Indian Ocean.

Key words : swordfish, *Xiphias gladius*, mtDNA, microsatellite loci, Indian Ocean

Introduction

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 1990's, peaking in 1998 at around 35,000 tons, and decreasing slightly over the recent five years. 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 (South West) using both mtDNA and microsatellite loci markers in order to validate protocols and to analyse the relatedness of the sampling locations.

Material and methods

A total of 90 samples were 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 was 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 are as follow : 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 1X 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 were 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). Significant departures from Hardy-Weinberg equilibrium were tested for the microsatellite data. For both mtDNA and microsatellite loci, differentiation between pairs of population 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) including 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 in each location (Table 1), the AMOVA revealed no genetic structure between the 4 populations (global Fst = 0.01) and that nearly all haplotypes variation was 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 to 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).

Discussion and conclusion

This preliminary study has 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 work 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) showed significant divergence between populations in other oceans. These studies analysed samples originating from various oceans and with sample sizes larger than 20 individuals.

This observation underlines the fact that the geographical scale and small sizes in the present study may not be large enough to detect differences that are present, and suggests the potential for confirmation by a larger sampling effort.

In conclusion, two approaches can be proposed for future works. First, larger sample sizes would increase likelihood of detecting differences using both mtDNA and nDNA, and may confirm the hypothesis of a unique stock in this region. Alternatively, as there are no obvious oceanographic boundaries between these sample points, the locations may comprise a single population which can serve as a single sample in future work extended to the whole Indian Ocean using more distant

sources, and including other biological (sex and individual size) and environmental (seasonal and oceanographic) parameters in order to better understand migratory behaviour of the swordfish. A combination of tagging, catch analysis, microchemistry, parasites analysis, and genetic techniques may provide fishery biologists with more precise information for managing swordfish in the Indian Ocean.

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Author addresses
C. J. : Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer) de La Réunion, Rue Jean Bertho, BP 60, 97 822 Le Port Cedex, Ile de La Réunion, France. E-mail: <claire.jean@ifremer.fr>
J. B. : Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer) de La Réunion, Rue Jean Bertho, BP 60, 97 822 Le Port Cedex, Ile de La Réunion, France. E-mail: <jerome.bourjea@ifremer.fr>
E. J. : Centre de Recherche et de coopération International en Recherche Agronomique pour le Développement (CIRAD), Laboratoire de pathologie et de génétique moléculaire, 7 chemin de l'Irat, 97410 Saint-Pierre, Ile de La Réunion, France. E-mail: <emmanuel.jouen@cirad.fr>
M. T. : Institut Français de Recherche pour l'Exploitation de la Mer (Ifremer) de La Réunion, Rue Jean Bertho, BP 60, 97 822 Le Port Cedex, Ile de La Réunion, France. E-mail: <marc.taquet@ifremer.fr>

Table 1. 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 characters).

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

Table 2. MtDNA. Pairwise comparisons of the four locations showing Fst (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	0.0017	*	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. Microsatellite loci. Allele statistics and sizes for each locus in each population.

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

Table 4. Microsatellite loci. Pairwise comparisons of the four locations showing Fst (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	*