Assessment of the stock structure of the Indian Ocean Swordfish (*Xiphias gladius*) : a proposal for a multidisciplinary study

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Abstract

After peaking at 35,000 metric tons in 1998, the swordfish catch began to decrease despite an increase of the fishing effort. This could be the consequence of a decrease of the swordfish biomass particularly in the western Indian Ocean. In 2004, the total swordfish catch was around 31,000 metric tons. In 2005, the Reunion longline fleet comprises 36 boats from 8 to 33 meters long, operating in the southwest Indian Ocean. With 1,000 metric tons in 2005, the swordfish catch of the Reunion fleet represents 3% of the whole Indian Ocean swordfish catch. Swordfish proportion in the catch which was 60% in 2000, decreased to 32% in 2005. As for many swordfish fisheries in the Indian Ocean, we have observed a decrease in the catch of the Reunion fishery since 1998 followed by a small increase in 2004 and 2005. The CPUE have also decreased significantly since the first years of the fishery but they lead to stabilize during the last years. On the other hand, the mean size of the swordfish caught by the Reunion fleet during the last 12 years has remained stable without any trend to decrease. On the basis of the stock indicators, the IOTC Scientific Committee concluded that the current level of catch is unlikely to be sustainable. Nevertheless, the assessment of this stock needs an increase in number and quality of fishing and biological data. The lack of knowledge on the swordfish stock structure and on the migratory behaviour limits the implementation of a sustainable management on this shared resource. A pilot study has been done on 90 swordfish samples collected in 4 southwest Indian Ocean areas. Analysis of mtDNA and 6 microsatellite loci showed a strong heterogeneity within populations. These results plead in favour of an unique stock in this region. However, the geographic scale and the sample size are probably inadequate to observe significant differences between these 4 areas. Following these preliminary results, we planned to build a new program at the whole Indian Ocean scale, with more sampling sites and samples. This program will be developed on a multidisciplinary approach including genetic, otolith microchemistry, breeding, and mercury contamination.

Keywords: Swordfish, Population structure, Genetic, Otolith, Mercury contamination, breeding

1 Swordfish fishery in the Indian Ocean and from Reunion Island

1.1 Swordfish fishery status in the Indian Ocean

After peaking at 35,000 metric tons in 1998, the swordfish catch began to decrease despite an increase of the fishing effort. This could be the consequence of a decrease of the swordfish biomass particularly in the western of the Indian Ocean. In 2004, the total swordfish catch was around 31,000 metric tons. On the basis of the stock indicators, the IOTC Scientific Committee concluded that the current level of catch is unlikely to be sustainable. Nevertheless, the assessment of this stock needs an increase in number and quality of fishing and biological data. The lack of knowledge on the swordfish stock structure and on the migratory behaviour limits the implementation of a sustainable management on this shared resource.

1.2 Reunion Swordfish fishery status

The first longline fishing test for large pelagic fish began in Reunion Island at the beginning of the nineties. From the beginnig of this fishery, the fleet comprises boats of different sizes operating on fishing grounds more or less distant from the island. In 2005, the fleet comprises 36 boats from 8 to 33 meters long, operating in the southwest Indian Ocean (Figure 1).

With 1,000 metric tons in 2005, the swordfish catch of the Reunion fleet represents 3% of the whole Indian Ocean swordfish catch. Swordfish proportion in the catch which was 60% in 2000, decreased to 32% in 2005. Yellowfin (*Thunnus albacares*), albacore (*Thunnus alalunga*) and bigeye (*Thunnus obesus*) are the 3 other main species with around 600 metric tons (20%) for each of them.

As for many swordfish fisheries in the Indian Ocean, we have observed a decrease in the catch of the Reunion fishery since 1998 followed by a small increase in 2004 and 2005 (Figure 2). This could be explained by a situation of fully exploited resource in the fishing ground of this fleet (FAO 51 : sub area 6 and 7). The CPUE have also decreased significantly since the first years of the fishery (Figure 3) but they lead to stabilize during the last years. On the other hand, the mean size of the swordfish caught by the Reunion fleet during the last 12 years has remained stable (Figure 4) without any trend to decrease. This is an important and positive factor because a decrease in the mean size would indicate at least a situation of local overfishing. The evolution of the swordfish mean size by quarter, calculated on the whole fishing period (Figure 5) could be the consequence of the breeding activity in this particular fishing area (Poisson et al. 2001).

Regarding the economic weight of this fishery for Reunion Island, it seems to be necessary to take management measures for this species. But the definition of the management measures needs an improvement of our knowledge on the biological characteristics of the swordfish as well as on the stock structure and dynamic of this species in the Indian Ocean.

2 Pilot study on the genetic structure of swordfish in the southwest Indian Ocean

Molecular techniques have been successfully developed to study stock structure and population dynamic of swordfish in the three oceans and in the Mediterranean. Recent genetic studies on mitochondrial DNA (mtDNA) (Kotoulas et al. 1995, Alvarado Bremer et al. 1996, Rosel et Block 1996, Chow et al. 1997, Reeb et al. 2000, Alvarado Bremer et al. 2005) and nuclear DNA (Chow et Takeyama 2000, Kotoulas et al. 2003, Reeb et al. 2003) have revealed at least four distinct stocks of swordfish in the world: Mediterranean, North Atlantic, South Atlantic and Indo-Pacific with a subtle structure in the Pacific (Reeb et al. 2000). No genetic heterogeneity has been reported between the Indian and Pacific Oceans (Chow et al., 1997), but genetic studies on swordfish remain insufficient in the 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.

2.1 Pilot study

A total of 90 samples were collected between February and May 2005 in 4 different fishing zones of the southwest Indian Ocean : Reunion Island (20), southern Madagascar (30), southwest of Madagascar (20), and Seychelles Archipelago (20). Muscle tissues of swordfish were taken using a sterilized scalpel, stored in ethanol

90% or 20% DMSO (Dimethyl Sulfoxide). DNA was extracted with Chelex 10% and DNA Animal Tissue Kit (Qiagen). PCR-RFLP method (Polymerase Chain Reaction - Restriction Fragment Length Polymorphism) defined by Chow et al. (1997) was used to analyse the control region (D-Loop) of the mtDNA, but only 2 endonucleases – *RsaI, AluI* – were used in the present study. Moreover, 6 microsatellite loci defined by Reeb et al. (2003) – *Xg-55, Xg-56, Xg-66, Xg-75, Xg-144, Xg-166* – were amplified and separated on a genetic analyser.

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 Fst (Wright, 1951). Population genetic structure was inferred using an Analysis of Molecular Variance approach (AMOVA) described in Excoffier et al. (1992) and by testing various alternative groupings.

2.2 Results and conclusions

MtDNA

A total of 86 samples were successfully amplified. Endonucleases digestions resulted in 7 restriction patterns in *RsaI* and 5 in *AluI* . Combining the restriction patterns of the two endonucleases yielded a total of 16 haplotypes, and 5 dominant haplotypes in all the individuals (Figure 6). Gene diversity within population was very high (>0.79) in the 4 locations. Moreover, repartition of the haplotype frequencies appeared highly complex in each location, with absent haplotypes in some sites and others highly represented in other sites. Despite these differences in haplotypes frequencies distribution, no significant differences were observed between populations, and indicated that most of the variability was contained within population. Pairwise comparison of the four locations showed very low values of Fst (0.0017 to 0.02) with non-significant P values (P>0.197; Table1), indicating that there is no significant differences between the populations. These results suggest the hypothesis of a single stock in this area.

Table 1. Pairwise comparisons of the 4 locations (Fst - below diagonal and P- above diagonal).

	Seychelles	La Réunion	South of Madagascar	Southwest of Madagascar
mtDNA				
Seychelles	*	0.37488	0.21785	0.19702
La Réunion	0.0017	*	0.34612	0.29190
South of Madagascar	0.01505	0.00423	*	0.23769
Southwest of Madagascar	0.02071	0.00818	0.01251	*
Microsatellites				
Seychelles	*	0.99902	0.99902	0.54492
La Réunion	-0.00199	*	0.99902	0.49609
South of Madagascar	0.00000	0.00000	*	0.14844
Southwest of Madagascar	0.00082	0.00080	0.00078	*

Microsatellite loci

A total of 71 samples were successfully 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). However, the number of genotypes was often similar to the number of individuals analysed. Observed genotype proportion in each population generally accorded well with Hardy-Weinberg equilibrium for 4 loci (P>0.05), but 2 loci significantly deviated from expectations (*Xg-55* and *Xg-75*, P<0.01) in all localities showing heterozygote deficits. These results suggest that the sampling is not large enough to draw final conclusions. However, except for *Xg-66*, the comparison of size range loci between samples showed variability, indicating that these microsatellites are adapted for swordfish stock discrimination in this area. As observed for mtDNA, pairwise comparison of the four locations did not show significant differentiation (Fst = [-0.002 ; 0.0008], P>0.14 ; Table1).

3 Projet multidisciplinaire

Currently over or fully exploited in some Indian Ocean areas, we need to improve the knowledge on the shared resource in order to implement a regional management plan. That is why Ifremer plans to develop an international project on swordfish stock structure and characteristic in the Indian Ocean. This 3 years scientific project will gather several partners from fishing and rim countries of this ocean. Motivated by the preliminary results on genetics in the southwest Indian Ocean, this program will be composed of 4 complementary components.

3.1 Genetic analysis

The Pilot study conducted by Ifremer in 2005 revealed no significant differentiation between populations, suggesting a unique stock of swordfish in the Southwest Indian Ocean. However, the small sample size and the high heterogeneity within populations found in this study lead to arduous statistical analyses and interpretations. These results strongly encourage to continue the study by increasing the sampling and integrating other biological and environmental parameters in order to validate or not the hypothesis of a unique stock in the Indian Ocean.

3.2 Microchemistry analysis of otolith

In order to better understand swordfish stock structure and migration in the Indian Ocean, a microchemistry analysis of otoliths can supplement the genetic study. Otoliths are biological markers which reflect the quality of the environments inhabited by the fishes during their life history. They have been qualified in recent literature as permanent recorders of the exposition to an environment (Campana et al. 1997). Otolith microchemistry is mostly used in stock discrimination (Campana 1999, Tresher 1999, Campana et al. 2000). Thus, this technique has been applied to stock identification and discrimination studies for species living in various habitats (Edmonds et al. 1989, Northcote et al. 1992, Thresher et al. 1994, Campana and Gagné 1995, Gillanders and Kingsford 1996, Campana 1999, Patterson et al. 1999, Jones et al. 1999, De Pontual et al. 2000, Eldson and Gillanders 2003, Chittaro et al. 2004, Bergenius et al. 2004, Patterson et al. 2004). In the case of the swordfish, this study can be used to analyse the variability in the otolith chemical compositions from different locations, and so, to complete genetic results.

3.3 Analysis of the rate of mercury contamination

Fish contamination by heavy metals, particularly mercury (Hg), has been more and more observed in all the fisheries of the world (De Sylva et al. 2000, Konstantogianni et al. 2003, Campbell et al. 2005, Morrissey et al. 2005). This contamination is due to the bioaccumulation properties of many metals which concentration increase with trophic levels. The relatively long life of billfishes, especially the swordfish, may predispose them to such bioaccumulation problems. This contamination may also have repercussions on the market and thus, on fisheries. Pelagic fisheries are very important in the Indian Ocean, and such marketing problems have already been observed by some ACP countries (Africa, Caribbean and Pacific). Hence, it seems essential to simultaneously describe the current situation in this ocean in the most global and the most detailed manner possible in order to better understand relations between contamination rate and biological and environmental parameters. To date, many countries have adopted health advisory limits with respect to these species and contaminants (Canada, USA, EU, etc…), limits that vary according to the species. However, as there is no available structured data for a geographic area covering the Indian Ocean, it is not yet possible, to discuss on the sanitary status of the swordfish and to adopt health advisories for this ocean. The integration of this component in the project will allow to get more data on the swordfish stock structure. In fact, heavy metals, such as mercury, are indicators of environment quality and can allow, as genetics and otolith microchemistry, to study the stock structure of swordfish in the Indian Ocean.

3.4 Biological analysis of reproductive organs

Knowledge on swordfish reproduction seems essential to understand the dynamic of this species and to take measures for its sustainability. The reproduction of a stock depends on the fertile biomass which has to be sufficient to renew the part taken by the fisheries. Knowledge on fertility and sex-ratio is necessary to evaluate the dimension of a stock. An analysis of the bibliography shows that few studies on reproduction have been conducted in the Indian Ocean (Poisson et al, 2001) whereas several studies have been conducted in various regions of the world (Nakano and Bayliff, 1992; Arocha and Lee, 1995; De la Serna et al., 1995; DeMartini et al., 2000).

Poisson et al. (2001) showed that the southwest Indian ocean is an intensive spawning area during the southern summer (from October to April). Moreover, data analysis revealed a “gradient” in the sex-ratio and in the length repartition which is related to latitude in the southern Indian Ocean. However, similar studies should be conducted in other parts of the Indian Ocean to have a global vision of swordfish reproduction, size and sex distribution in this ocean. And regarding the intensification of swordfish fisheries and the first sign of over-exploitation of the stock, such studies on ecological and biological parameters seem to be very important for stock assessments and sustainable management measures.

3.5 Implementation of the research project

At present time, this international research project is at a preliminary stage. We need now to gather the potential partners of this project. In the first step, the scientific protocols and a regional sampling strategy have to be defined. In a second step, each partner will quantify its participation and implication in this project. It is the aim of the workshop organized by Ifremer in Reunion Island from 11 to 16 of September 2006. The agenda, details on organisation and accommodation could be obtain by e-mail at the following address: info.swordfish@ifremer.fr.

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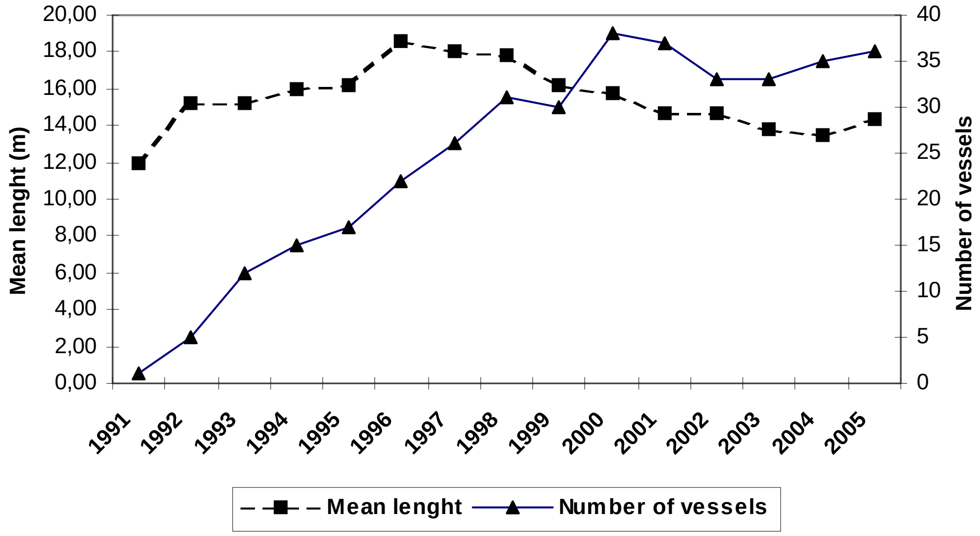

Figure 1: Evolution of the number and the mean size of the Reunion longliners since the beginning of the fishery.

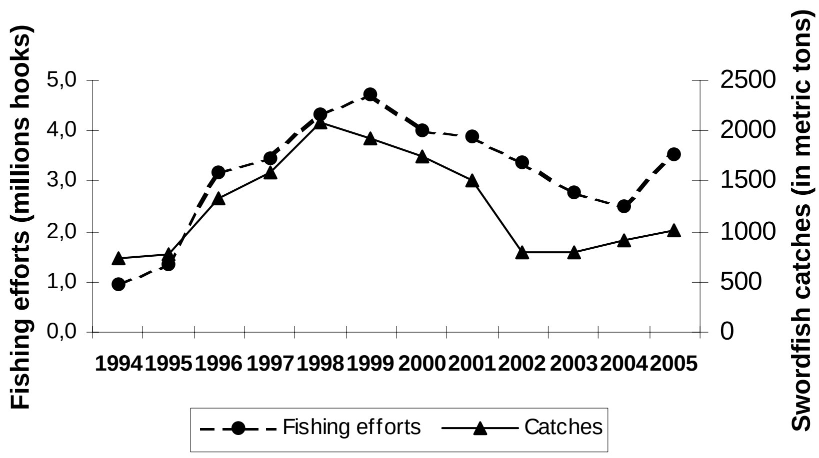

Figure 2: Evolution of the fishing effort and catches between 1994 and 2005.

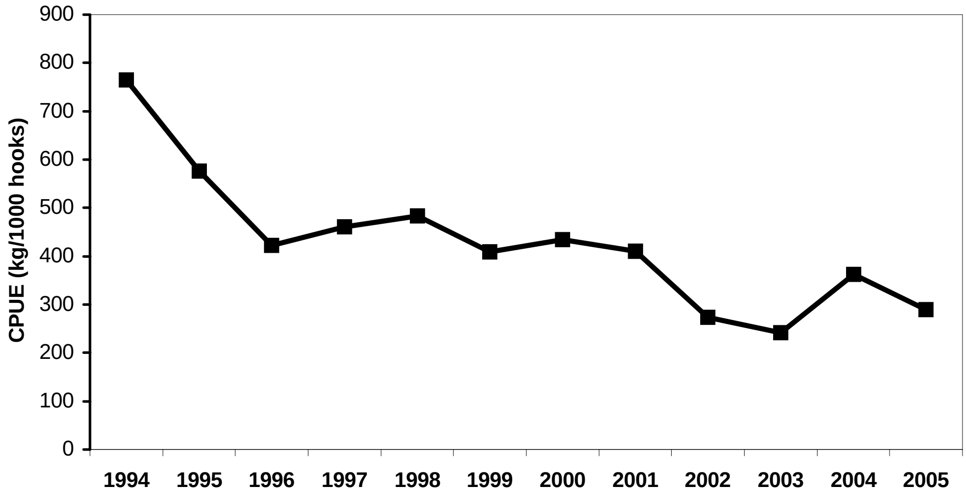

Figure 3: Evolution of the CPUE between 1994 and 2005.

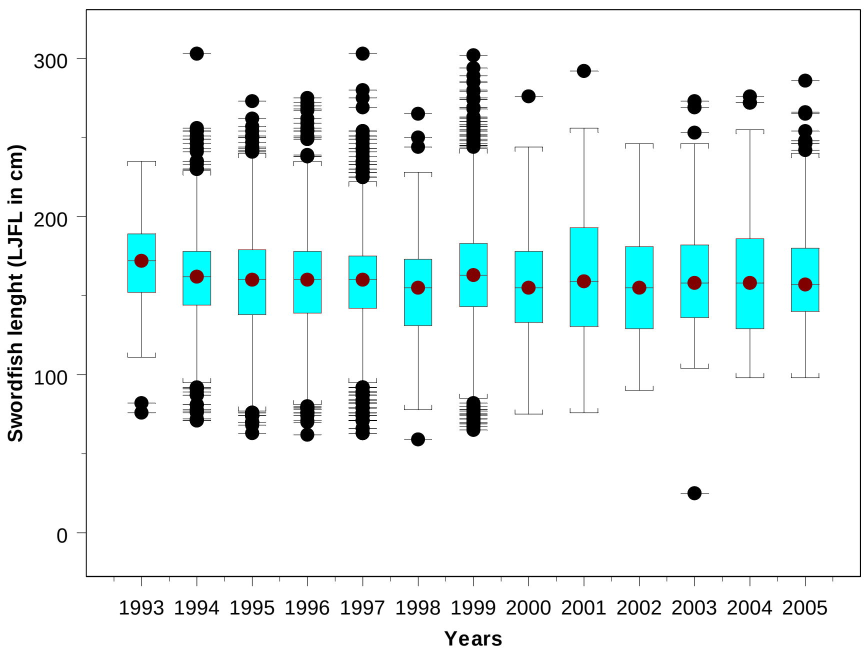

Figure 4: Evolution of the swordfish mean size (LJFL) between 1993 and 2005.

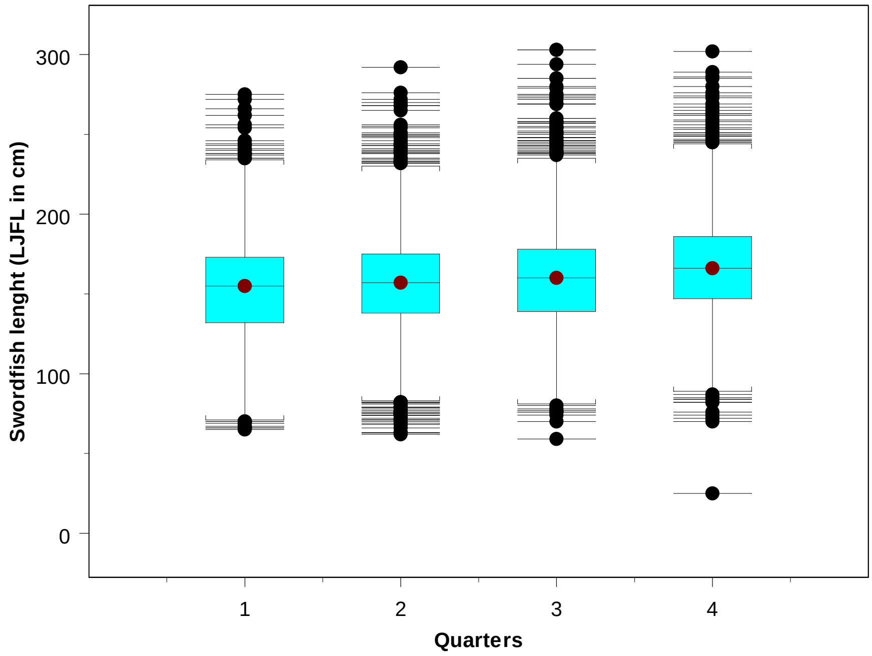

Figure 5 : Evolution of the swordfish quarter mean size (data from 1993 to 2005).

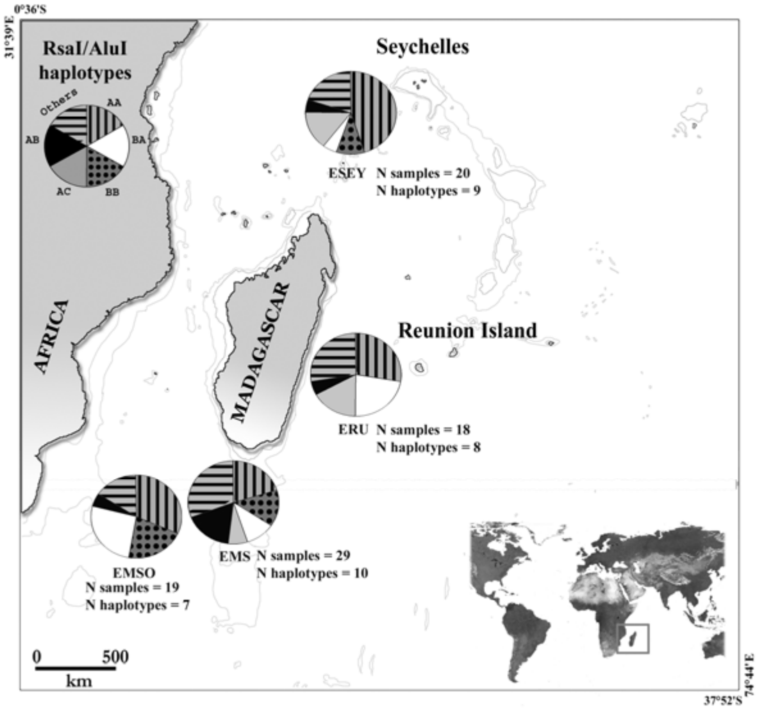

Figure 6: Repartition of the haplotype frequencies by locations (ESEY= Seychelles ; ERU= Reunion ; EMS= Madagascar ; EMSO= Southwest of Madagascar ; N=Number).

IOTC Working Party on Billfish

Colombo Sri Lanka, March 2006

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Ifremer

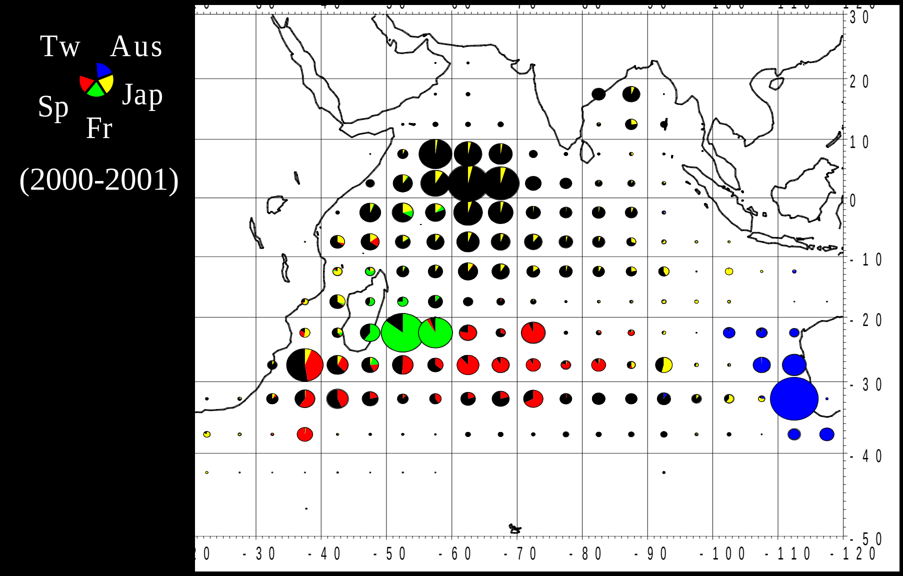
Exploitation of swordfish in the Indian Ocean

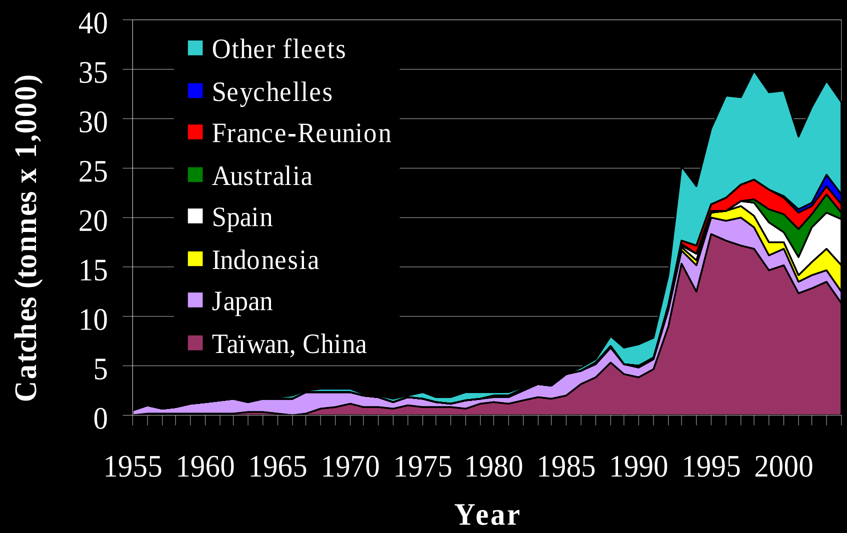
Swordfish = target or by-catch species of longline fisheries

Increase of catches until 1998 : peak at 35,000 tons

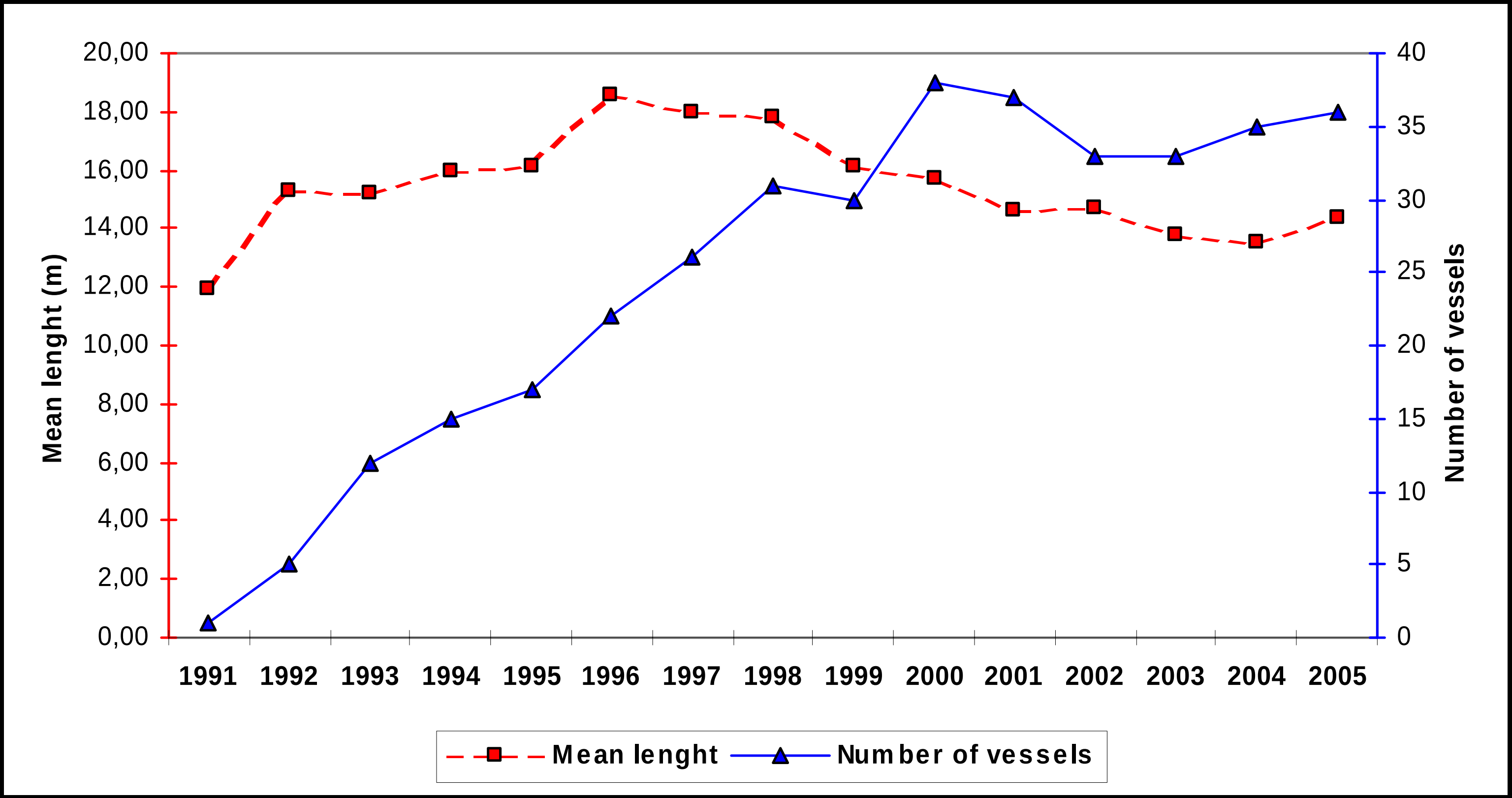
But global decrease over the recent 6 years, despite constant increase of effort

Highest catches in the WIO



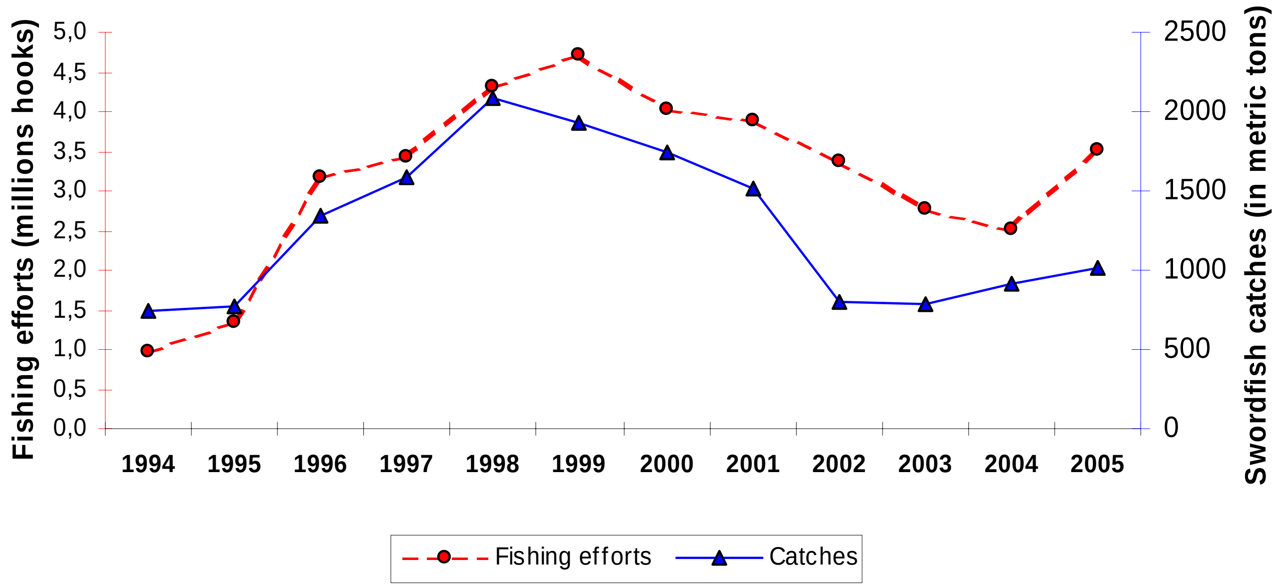


Reunion swordfish longline fishery



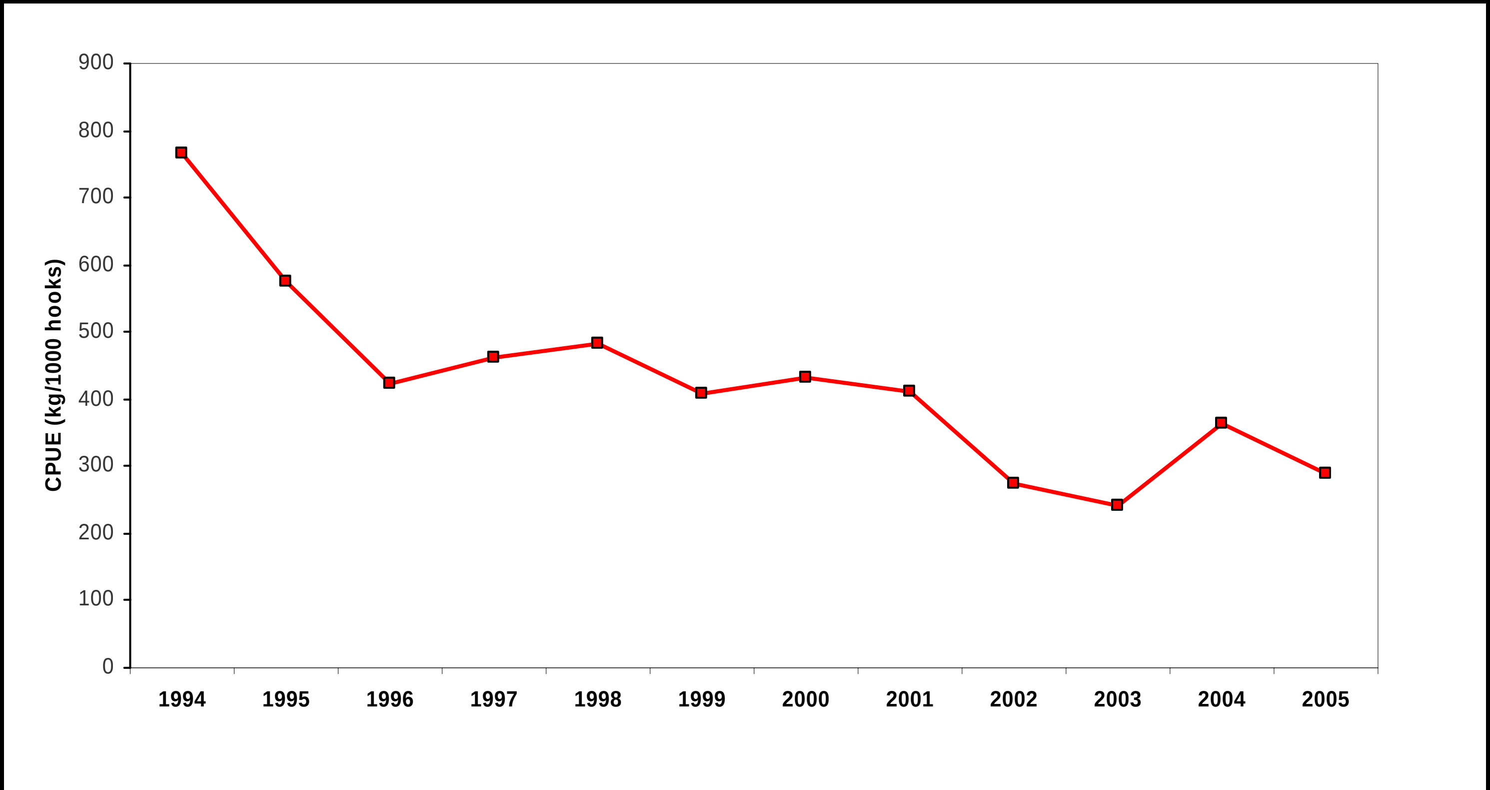
Evolution of the number and the mean size of the Reunion longliners since the beginning of the fishery

Reunion swordfish longline fishery



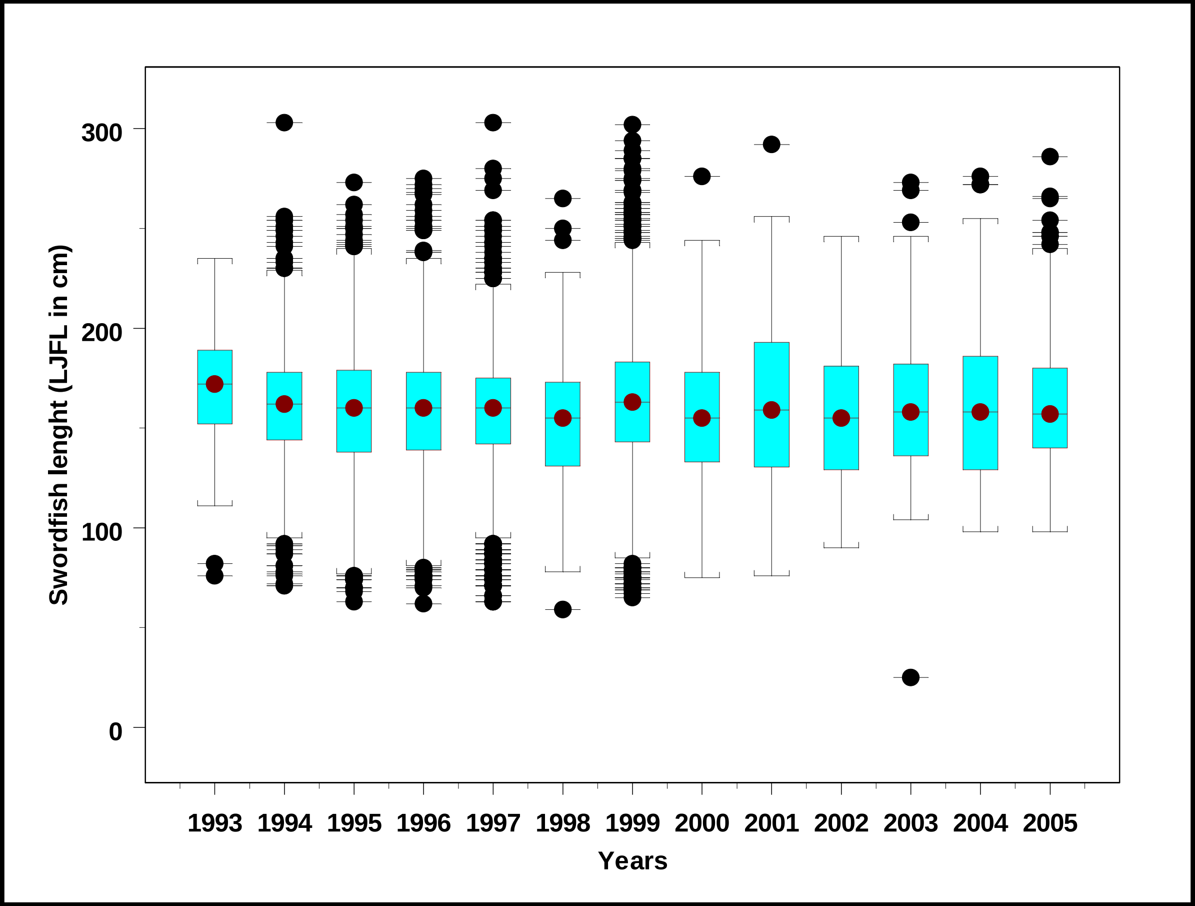
Evolution of the fishing effort and catches between 1994 and 2005

Reunion swordfish longline fishery



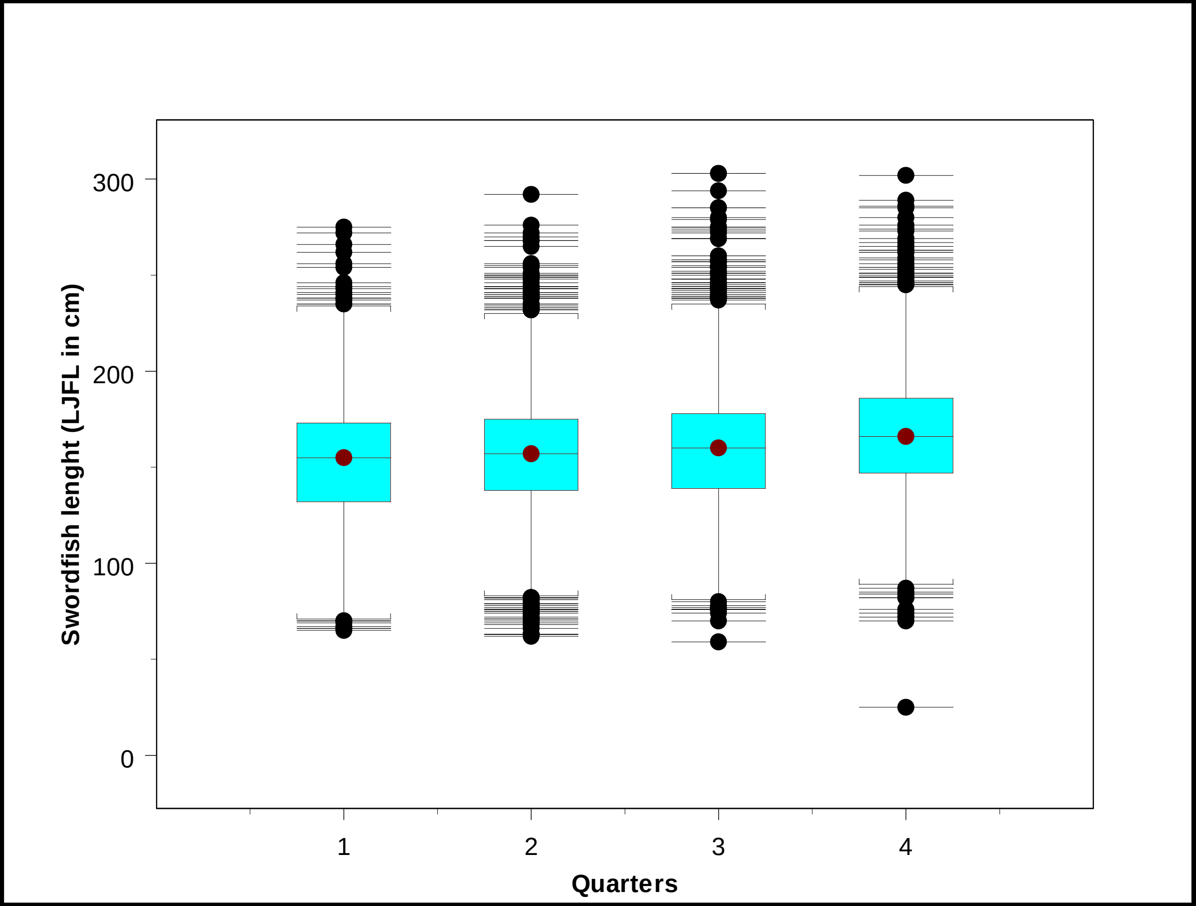
Evolution of the CPUE between 1994 and 2005

Reunion swordfish longline fishery



Evolution of the swordfish mean size (LJFL) between 1993 and 2005

Reunion swordfish longline fishery



Evolution of the swordfish quarter mean size (data from 1993 to 2005)

IOTC conclusions and recommendations

- Current level of catch (~32,000 t) unlikely to be sustainable

- Suspicion of overfishing in the SWIO

- But, currently no evidence of any declines in average size of the fish in the catch

<u>However, to perform swordfish stock assessment we need to</u>

⬇

Get more and better quality data on swordfish exploitation and biology

Improve our knowledge on the swordfish stock structure in the IO

Preliminary study on swordfish genetic structure in the SWIO

We tested molecular markers in SWIO

We got a first overview of the swordfish genetic characteristics in this restricted area

In order to

Build a larger project aiming to improve knowledge on the swordfish stock structure in the whole Indian Ocean

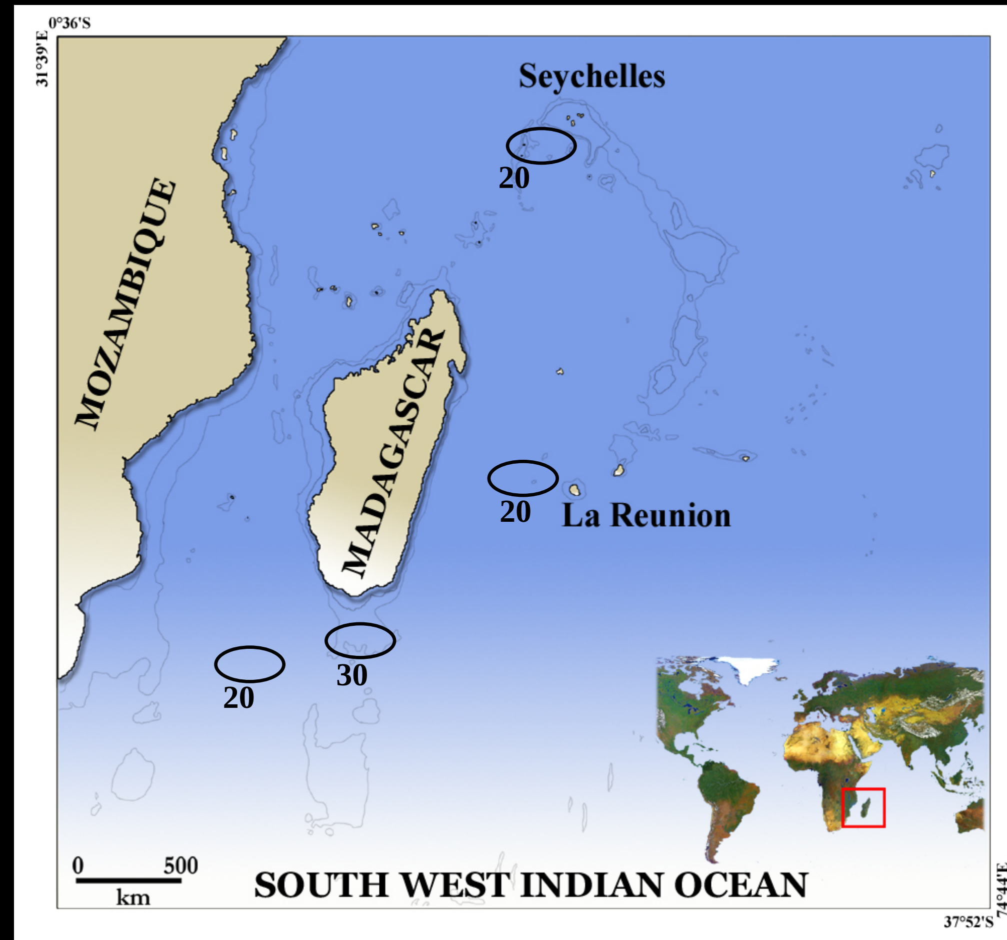
Preliminary study on swordfish genetic structure in the SWIO

90 samples in 4 sites :

Seychelles, La Reunion, Southern Madagascar, Southern Mozambique Channel

Total DNA extracted from muscle tissue with Chelex

PCR-RFLP analysis on mtDNA *(Chow et al., 1997)* with *RsaI* and *AluI*



Analysis of 6 microsatellite loci *(Reeb et al., 2003)*

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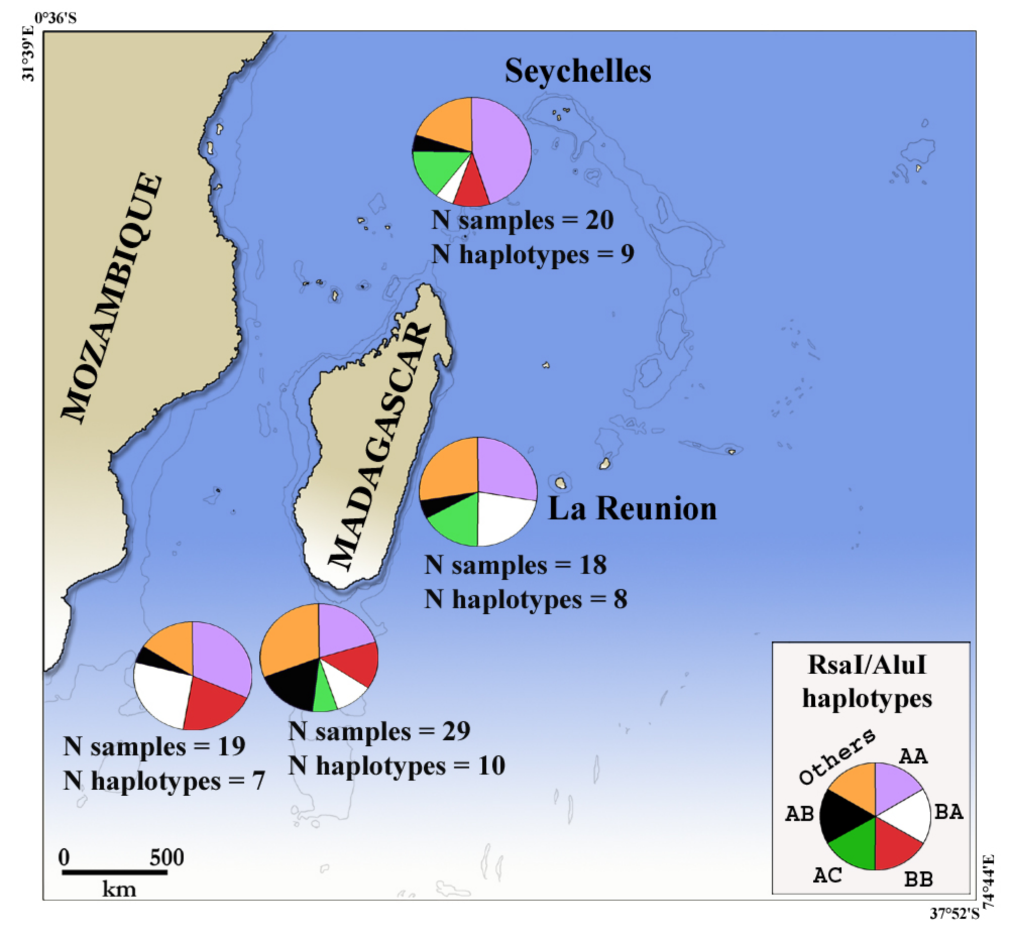
<u>mtDNA results</u>

Very high intra-population genetic diversity (>0.79)

Complex repartition of haplotype frequencies between sites

Very low *Fst* (<0.02) and non significant P values (>0.197)



- No significant differences between populations
- Problem of sample size

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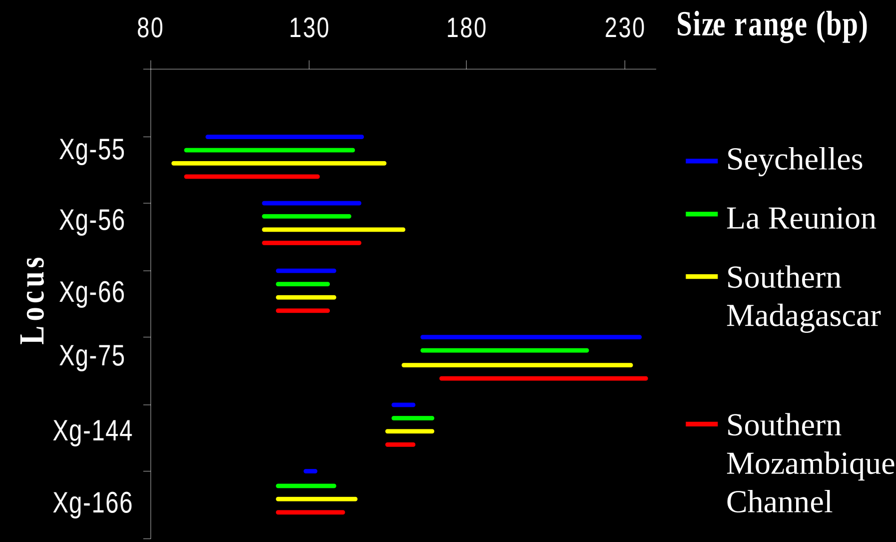
<u>Microsatellite loci results</u>



Number of alleles per locus highly variable [6;32], very high for *Xg-75* and *Xg-55* (32 and 26)

Xg-55 and *Xg-75* deviated from HW expectations

Locus by locus, size ranges differ between sites, except for *Xg-66*

Very low *Fst* (<0.0008) and non significant P values (>0.14)

→ - No significant differences between populations
- Problem of sample size

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Conclusions

Variability intra- (high) and inter- (low) populations

No significant differences between populations neither with mtDNA nor with microsatellite loci

Imbalance between geographic scale and sample size : too small

→ Necessary to enlarge the sampling and the geographic scale of the study

Ifremer

Multidisciplinary project on swordfish stock structure and characteristics in the IO

Motivated by :

- the situation of swordfish stock in the IO
- the lack of knowledge on swordfish stock structure and biology in the IO
- the preliminary study results
- economic importance for longline fisheries

4 complementary components based on a common pool of samples :

- To take advantage of the same sampling in order to collect various types of samples and to combine multiple topics

Multidisciplinary project on swordfish stock structure and characteristics in the IO

<u>1. Genetics :</u>

- To assess the heterogeneity between IO populations

With more samples distributed in a wider area (IO), mitochondrial and nuclear markers, biologic (sex, size) and seasonal data

<u>2. Otoliths microchemistry analysis :</u>

- To supplement and validate genetic data on stock structure :

Otoliths are permanent recorders of the exposition to an environment

- To get more information about migrations in the IO

Multidisciplinary project on swordfish stock structure and characteristics in the IO

3. Mercury concentrations analysis :

- To discriminate stocks
- To make an assessment of mercury contamination (/length, sex, localisation)
- To discuss on the sanitary status of the swordfish

4. Reproductive organs analysis :

- To improve knowledge on spawning areas of the swordfish in the IO, using stage of maturity and histological data
- To combine these data with stock structure and migration data

Multidisciplinary project on swordfish stock structure and characteristics in the IO

Project at a preliminary stage :

International workshop with all the partners

(http://www.ifremer.fr/drvreunion/)

Definition of sampling design and protocols

Drafting of the research program

Thank you!