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Otolith shape as a valuable tool to evaluate the stock structure of swordfish *Xiphias gladius* in the Indian Ocean

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Abstract :

Swordfish *Xiphias gladius* is an oceanic-pelagic species. Its population structure in the Western Indian Ocean was studied from the shape of the sagittal otoliths of 391 individuals collected from 2009 to 2014. Normalised elliptical Fourier descriptors (EFDs) were extracted automatically using TNPC software. Principal components analysis (PCA) conducted on EFDs showed no significant effect of side (i.e. left or right otolith). Consequently, all 391 sagittal otoliths were used to identify stocks among six geographical areas: Reunion Island, Mozambique Channel, Rodrigues, South Africa, Madagascar South and Sri Lanka. To investigate the effects of sex, sampling year, sampling season, lower jaw fork length or geographical area on variations in otolith shape, redundancy analyses (RDAs) with permutation tests were conducted. The first four were non-significant (respectively, $p = 0.124$, $p = 0.721$, $p = 0.197$, $p = 0.463$), but geographical area appeared to discriminate groups significantly ($p < 0.05$). Furthermore, linear discriminant analysis (LDA) was performed and overall jackknife classification success reached 30%. Finally, a cluster analysis was conducted using Ward's hierarchical algorithm, which discriminated three different groups. However, each group consisted of individual samples from all geographical areas. In conclusion, our results were unable to identify a clear geographical separation of swordfish at the Indian Ocean scale, corroborating recent genetic studies in this region.

Keywords : connectivity, Fourier descriptors, morphometric markers, sagittal otoliths, stock discrimination

Introduction

Swordfish *Xiphias gladius,* Linnaeus 1758 is a highly migratory oceanic species, currently fully exploited by several commercial fisheries in the Indian Ocean either as a target species (e.g. European Union longline fleet) or a bycatch product (e.g. Taiwanese longline fleet). The spatial structure of large pelagic species is difficult to understand due to their wide geographic distribution, large population size, high fecundity and ability to migrate between oceans (Nakamura 1985). Consequently it is difficult to discriminate among subpopulations based on differences in life-history characteristics, and hence it is similarly difficult to divide populations into stock units that are defined by growth and renewal rates pertinent to geographic areas where fisheries are undertaken (Cadrin et al. 2014). On the basis of the most recent swordfish stock assessment in the Indian Ocean (IOTC 2015), levels of catches in the Indian Ocean as a whole for the period 2005–2014 (22 174 t – 35 693 t) were considered to be below the estimated maximum sustainable yield (MSY; 39 400 t). However since 2013 and on the basis of the fishery data and some structured stock assessment trials, the scientific committee of the Indian Ocean Tuna Commission (IOTC 2013) requested a population structure to be considered, as the South-West Indian Ocean (SWIO) may host an independent stock. The stock assessment clearly emphasises the importance of better knowledge of the spatial structure of this species at the scale of the Indian Ocean, and its relation with adjacent oceans (i.e. the Atlantic and the Pacific).

Spatial-structure information provides a basis for understanding fish population dynamics and contributes to reliable resource assessment for fishery management (Reiss et al. 2009). Several techniques may be used to identify stock limits, such as tagging experiments, analyses of spatial and temporal variation of genetic or morphometric markers, differentiation of life-history variables, parasites and contaminant concentrations (Pawson and Jennings 1996; Cadrin et al. 2014) or parasite composition (Garcia et al. 2011). Previous genetic studies identified some differentiation of swordfish in the Indian Ocean, including the Western Indian Ocean (Jean et al. 2006; Lu et al. 2006; Muths et al. 2009; Bradman et al. 2011), but conclusions on stock structure were inconsistent as a result of the marker used, the absence of stratification of sampling in space and time and the lack of information on reproductive behaviour. The reproductive biology of swordfish in the Indian Ocean is not well known. Three spawning grounds have been identified: the Gulf of Bengal, the Somalia coast where spawning takes place after April, and La Réunion where spawning is believed to take place from October to November (Yabe et al. 1959;

Palko et al. 1981; Mejuto et al. 2006; Poisson and Fauvel 2009). The existence of these grounds might explain differences between the conclusions of previous studies investigating the genetic stock structure of swordfish. A recent global study on population genetics using a multi-genetic marker approach and spatio-temporal analysis based on 2 231 individuals suggested there is a single panmictic population (i.e. a single stock) of swordfish in the Indian Ocean (Muths et al. 2013). However, that study also underlined the need for other stock discrimination approaches to investigate further the stock structure of this species, particularly in the Indian Ocean.

In the present study, swordfish stock structure was investigated using the shape of sagittal otoliths. Otolith morphology is influenced by both biotic and abiotic factors (Cardinale et al. 2004; Capoccioni et al. 2011). Analysis of the outline of otoliths has previously been used for stock discrimination purposes (haddock *Melanogramus eaglefinus*: Begg and Brown 2000; cod *Gadus morhua*: Galley et al. 2006; Petursdottir et al. 2006; Stransky et al. 2008; striped red mullet *Mullus surmuletus*: Benzinou et al. 2013). Based on the logistics and sample collection presented in Muths et al. (2013), with additional samples from South Africa, this study focuses on individual swordfish sampled over a wide geographic area, from South Africa to Sri Lanka. Otolith shape analysis was performed to discriminate components of the Indian Ocean swordfish stock.

Material and methods

Sample collection

Swordfish samples from South Africa to Sri Lanka (Figure 1; Table 1) were collected during the period 2009–2014 by observers on board commercial fishing vessels or at landing sites, and associated fishing data were recorded. Samples for this study were obtained from the same swordfish as those used for the genetic study by Muths et al. (2013). With the exception of South Africa, samples were collected over two years (2009–2010) and following a strategy targeting two distinct seasons – April–July, considered to be the non-spawning season, and September–February, thought to be the spawning season (Poisson and Fauvel 2009). The specimens from South Africa were collected during December 2013 and November 2014. Seasons were defined based on the limited information available on swordfish reproductive condition in the Indian Ocean, but could also be considered broadly as the austral winter and summer, respectively. Information on sampling location (latitude and longitude or 5° square) was noted systematically and the swordfish were classified according to the six sampling areas listed in Table 1. For consistency, we grouped the data as proposed by Muths et al. (2013). Sex and maturity (i.e. immature or mature) were determined by macroscopic examination of gonads. Only mature fish were included in this study to minimise the potential effect of sexual maturity, which may lead to a change otolith shape (Cardinale et al. 2004). Sagittal otoliths were extracted from a total of 391 individuals ranging from 56 cm to 300 cm lower jaw fork length (LJF) (mean 155.0 cm; SD 38.6).

Otolith shape analysis

Swordfish otoliths present a large dorsal area, between the excisura major and the excisura minor*,* which is very distinct from the antirostrum (Figure 2). Images of whole left and right sagittal otoliths were scanned (Epson V750) under reflected light and stored at high resolution (3 200 dpi). Image processing was performed using the image analysis system TNPC 7 ('Digital Processing for Calcified Structures'; www.tnpc.fr) with the sulcus acusticus facing upwards. In order to compare left and right otolith shapes, a mirror image of left otoliths was used.

Otolith length and width were measured (Figure 2) and the contour of each otolith was extracted using the automatic threshold in TNPC. To describe otolith contours, elliptic Fourier analysis (EFA; Lestrel 2008) was carried out. For each otolith, the first 99 elliptical Fourier harmonics (HI) were extracted and normalised with respect to the first harmonic using TNPC, rendering them invariant to otolith size, rotation and starting point of the shape measurements (Kuhl and Giardina 1982). To determine the number of harmonics required to reconstruct the otolith outline, the Fourier power (PF) was calculated for each individual otolith *k* as a measure of the amount of contour rebuilt by each harmonic:

$$PF(n_k) = \sum_{\mathrm{HI}=1}^{n_k} \frac{A_{\mathrm{HI}}^2 + B_{\mathrm{HI}}^2 + C_{\mathrm{HI}}^2 + D_{\mathrm{HI}}^2}{2}$$

where A_{HI}, B_{HI}, C_{HI} and D_{HI} are the parameters of the $\mathrm{HI}^{\mathrm{th}}$ harmonic and n_k is the total number of harmonics included. The value of n_k was chosen such that PF(n_k) explains 99.99% of the variance in contour coordinates, i.e. enabling the shape to be reconstructed at an accuracy of 99.99% (Lestrel 2008).

Statistical analyses

The difference in shape between left and right otoliths was tested for 91 individuals. Firstly, principal components analysis (PCA) was applied to a matrix of selected elliptical Fourier descriptors (EFDs) of otolith contours (EFDs as columns and individual otoliths as rows) (Rohlf and Archie 1984) and a subset of the resulting principal components was selected as otolith-shape descriptors according to the broken-stick model (Legendre and Legendre 2012). Secondly, partial redundancy analysis (pRDA) was modelled on the selected principal components (PCs) matrix, with otolith side (left/right) as the potentially influential variable and the individual as the conditioned variable. To visualise differences in otolith shape between right and left sides, an average otolith shape for the group from each side was rebuilt based on average EFDs.

The effects of the variables LJF (cm), sex, sampling year, sampling season and sampling area were tested on 391 individual samples (300 images of right otoliths and 91 mirror images of left otoliths were combined) using an RDA, where the explained matrix was combined with permutation tests on the selected PC matrix from the PCA, and the explanatory matrix consisted of the tested effects and the interactions between them. For each factor, the sample groups consisting of <10 individuals were removed before the analyses. To test the significance of each explanatory variable, a permutation test (marginal effect, Type II) was used (Legendre and Legendre 2012).

To discriminate fish classified according to the six sampling (fishery) areas, based on their otolith shapes, a linear discriminant analysis (LDA) with jacknifed prediction was applied to the selected PC matrix (Rencher and Christensen 2012). To evaluate the resulting discriminant functions, the percentage correct classification of individuals to sampling area was calculated using jacknife cross-validation and Wilk’s lambda criteria (Klecka 1980). Finally, a cluster analysis (Ward's hierarchical algorithm based on squared Euclidean distances) was performed on the selected matrix of EFDs to classify individuals with similar otolith shapes.

Statistical analyses were performed using the ‘Vegan’ (Oksanen et al. 2013), ‘MASS’ (Venables and Ripley 2002), ‘CAR’ (Fox and Weisberg 2011), ‘FactoMinR’ (Lê et al. 2008), ‘HH’ (Heiberger and Holland 2004) and ‘Ellipse’ (Murdoch and Chow 1996) packages in the statistical environment R (R Core Team 2014).

Results

Among the 99 Fourier harmonics extracted to describe otolith contours from left and right otoliths of 91 individuals, the first 39 harmonics explained at least 99.99% of the otolith variation and were thus used for the multivariate analysis. Only the first seven PCs were significant, as determined by their eigenvalues exceeding the threshold eigenvalue generated by the broken-stick model. The pRDA combined with permutation tests showed no significant difference between the left and right otolith shape (p = 0.143). This was corroborated by the comparison of average otolith shape of the group from each side based on average EFDs (Figure 3).

As the left and right otoliths were comparable, 300 images of right otoliths and 91 mirror images of left otoliths were combined. PCA of the first 39 Fourier harmonics showed that the first and the second PCs accounted for 35.5% and 24.8% of the total variance, respectively. Only the first seven PCs were significant, as determined by their eigenvalues exceeding the threshold eigenvalue generated by the broken-stick model. The effect of LJF ($p = 0.463$), sex ($p = 0.124$), sampling year ($p = 0.721$), sampling season ($p = 0.197$) and sampling area ($p = 0.002$) were tested using an RDA. The interaction between sampling year and sampling season was not significant ($p = 0.693$). The relationship between the ratio of otolith length to otolith width and the LJF of fish showed that the size effect was not significant ($p > 0.05$).

Only the effect of sampling area was significant ($p < 0.05$), and hence sampling area was used as an explanatory variable in the subsequent LDA. The overall jacknifed classification success was 30% (Table 2). The analysis showed significant differences among groups of swordfish sampled in different areas of the Indian Ocean (Wilks' $\lambda = 0.017$, $F = 1.255$, $p = 0.001$). The misclassification percentage for each sampling area was explained by all other areas and not only adjacent areas (Table 2). The hierarchical cluster analysis performed on the matrix of 39 Fourier harmonics identified three clusters of fish (Figure 4). The three clusters each consisted of individuals from all sampling areas (Table 3).

Discussion

The genetic study by Muths et al. (2013) identified differences between Atlantic and Indian Ocean swordfish stocks but did not investigate the possibility of subdivisions in Indian Ocean stock(s). Otolith-shape analysis is another efficient stock-identification tool linked to genetic heterogeneity and the influence of environmental factors (Campana and Casselman 1993; Cadrin and Friedland 1999; Torres et al. 2000; Cardinale et al. 2004; Swan et al. 2006; Vignon and Morat 2010). The external contour of otoliths can be described by several techniques such as univariate descriptors (coefficient of form, roundness, circularity, etc.), geometric morphometric analyses (Ponton 2006; Ramirez-Perez et al. 2010; Vergara-Solana et al. 2013), wavelet functions (Parisi et al. 2005; Sadighzadeh et al. 2014), growth markers (Benzinou et al. 2013) and the geodesic method (Benzinou et al. 2013). Among these, the elliptical Fourier analysis remains the most powerful and widely used method to describe otolith external shape (Agüera and Brophy 2011; Capoccioni et al. 2011; Ferguson et al. 2011; Legua et al. 2013; Paul et al. 2013). The method has the advantage of being unaffected by short-term changes in fish condition (Campana and Casselman 1993) resulting from environmental variation (Campana 1999). Nevertheless, its biological interpretation is more complex than in the case of methods based on linear morphometric descriptors (Stransky and MacLellan 2005). Some studies have combined the elliptical Fourier analysis and some basic descriptors of otolith contours to aid interpretation of results (Campana and Casselman 1993; Begg and Brown 2000; Galley et al. 2006; Merigot et al. 2007; Fergusson et al. 2011; Benzinou et al. 2013; Legua et al. 2013). This approach has provided relevant stock-structure information on demersal species such as the striped red mullet in the North-East European region (Benzinou et al. 2013). No study of stock identification using otolith shape has yet been undertaken on large migratory and long-lived tropical pelagic species.

In our study, prior to analysis of the geographical effect on otolith shape, some other factors known to affect shape were tested in order to exclude potential confounding factors (Vignon and Morat 2010). Firstly, no effect of side was observed, i.e. there was no significant difference between the left and the right otolith shape. A similar result was obtained for another highly migratory oceanic species, the bluefin tuna *Thunnus thynnus*, in an analysis of otolith morphological characteristics (Megalofonou 2006).

Sampling in this study was restricted to adult fish to eliminate the effect of sexual maturity, which has the potential to modify the outline contour of otoliths (Campana and Casselman 1993; Cardinale et al. 2004). The effect of LJF, sex and sampling year were not significant. The absence of a sexual dimorphism effect on otolith shape of swordfish has also been shown for several other species, e.g. Atlantic mackerel *Scomber scombrus* (Castonguay et al. 1991), haddock (Begg et al. 2000), lake trout *Salvelinus namaycush* (Simoneau et al. 2000), Atlantic cod (Cardinale et al. 2004) and blue whiting *Micromesistius poutassou* (Mahé et al. 2016). However, in other species, e.g. herring *Clupea harengus* (Bird et al. 1986), orange roughy *Hoplostethus atlanticus* (Gauldie and Jones 2000) and southern blue whiting *Micromesistius australis* (Legua et al. 2013), there was a significant effect of sex on otolith shape. These authors attributed this to somatic growth rate differences between sexes. However, if female swordfish in the Indian Ocean grow faster and attain a larger maximum body size than males, as is the case in the Pacific Ocean (Sun et al. 2002; De Martini et al. 2007), there would be no significant effect of somatic growth on otolith growth. Sampling year had no significant effect on the swordfish under study, which corroborates studies on species such as Atlantic salmon *Salmo salar* (Friedland and Reddin 1994) and Atlantic cod (Campana and Casselman 1993). Large pelagic fishes are also known to undertake extensive oceanic migrations from spawning areas to foraging grounds (e.g. bluefin tuna; Rooker et al. 2007). We found that otolith shape of swordfish did not differ significantly during and outside the spawning season, indicating that otoliths of foraging and spawning swordfish are of the same shape.

Finally, the RDA revealed a sampling-area effect on otolith shape of swordfish ($p < 0.05$). This was confirmed by LDA, which indicated significant differences among groups of swordfish sampled in different fishery areas of the Indian Ocean. However, the misclassification percentage of the cross-validation was 70%. Moreover, these specimens were not associated with adjoining sampling areas but with all sampling areas, regardless of location in the Indian Ocean. Thus, no geographical substructures could be distinguished in the Indian Ocean swordfish stock. Large geographic migrations might be an explanation for poor allocation success among geographic areas in this study, particularly if there are feeding aggregations composed by a mixture of multiple spawning groups. The cluster analysis supports this notion, with three groups each comprising fish from all sampling areas. Possible sources of misclassification in otolith-shape analysis are individual variability and migration (Campana and Casselman 1993; Tracey et al. 2006).

Swordfish migration patterns are among the most complex of the pelagic species and are known to be highly variable, with one individual reported to have travelled 3 053 km during a period of 90 days while another remained within 21 km of the tagging location during the same period (Palko et al. 1981; Sedberry and Loefer 2001). The Indian Ocean is divided between two hemispheres and comprises marine ecosystems that vary greatly across latitudes (Sherman and Hempel 2008). Major oceanographic features include the westward-flowing South Equatorial Current and, at around 12° S, the hydrochemical South Tropical Front, which separates two large oligotrophic areas, the Indian Monsoon Gyre Province in the north and the Indian South Subtropical Gyre Province (Longhurst 1998; Schott et al. 2009). However, swordfish is a highly mobile pelagic species with, for example, one individual recaptured 6 670 km to the south-east of the point of release in the Indian Ocean (Kadagi et al. 2011). Temperature and oxygen are the main environmental factors that control the migration and distribution of large pelagic fishes (Carey 1990; Brill 1994; Prince and Goodyear 2006; Sund et al. 2006). Swordfish appears to be very highly tolerant of variations and extremes of temperature and oxygen and its movement patterns are controlled largely by resource availability and reproduction (Dewar et al. 2011). Consequently, the hydrochemical South Tropical Front does not seem to be a barrier to movement of this species and it seems that there is substantial connectivity between the north and south of the Indian Ocean.

Conclusion

Swordfish is a highly migratory oceanic species, currently fully exploited by several commercial and recreational fisheries in the Indian Ocean. Our results are consistent with those of a recent genetic-based study of swordfish in the Indian Ocean by Muths et al. (2013), in that clear geographic separation of subpopulations could not be identified. Future research should investigate patterns of otolith shape of swordfish sampled in the South-East Atlantic, to elucidate connectivity between the Atlantic and Indian oceans. As previously suggested for the bigeye tuna *Thunnus obesus* (Durand et al. 2005) and for swordfish (Muths et al. 2013), it would be of interest to investigate patterns of habitat use between the two oceans by large pelagic fishes in South African waters.

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Figure legends

Figure 1: Map of location of swordfish samples collected between 2009 and 2014 in the Indian Ocean. The rectangles indicate sampling areas (1: La Réunion, 2: Mozambique Channel, 3: Rodrigues, 4: South Africa, 5: Madagascar South and 6: Sri Lanka)

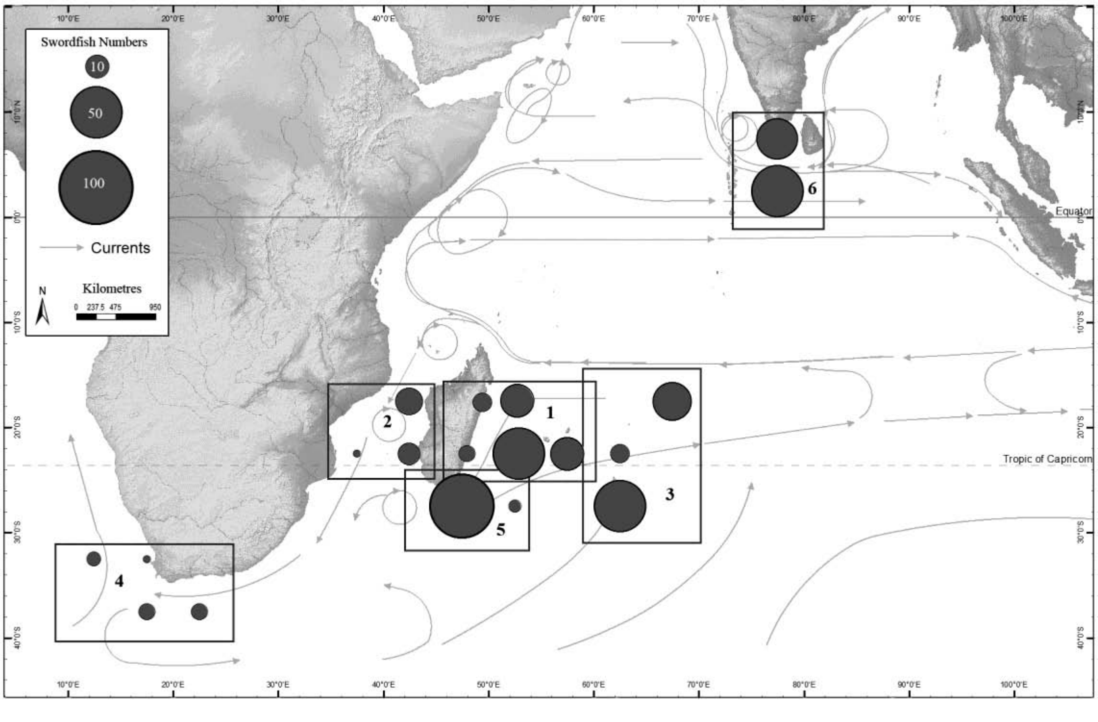

Figure 2: Photograph of a whole swordfish sagittal otolith showing terminology and measurements taken during the study

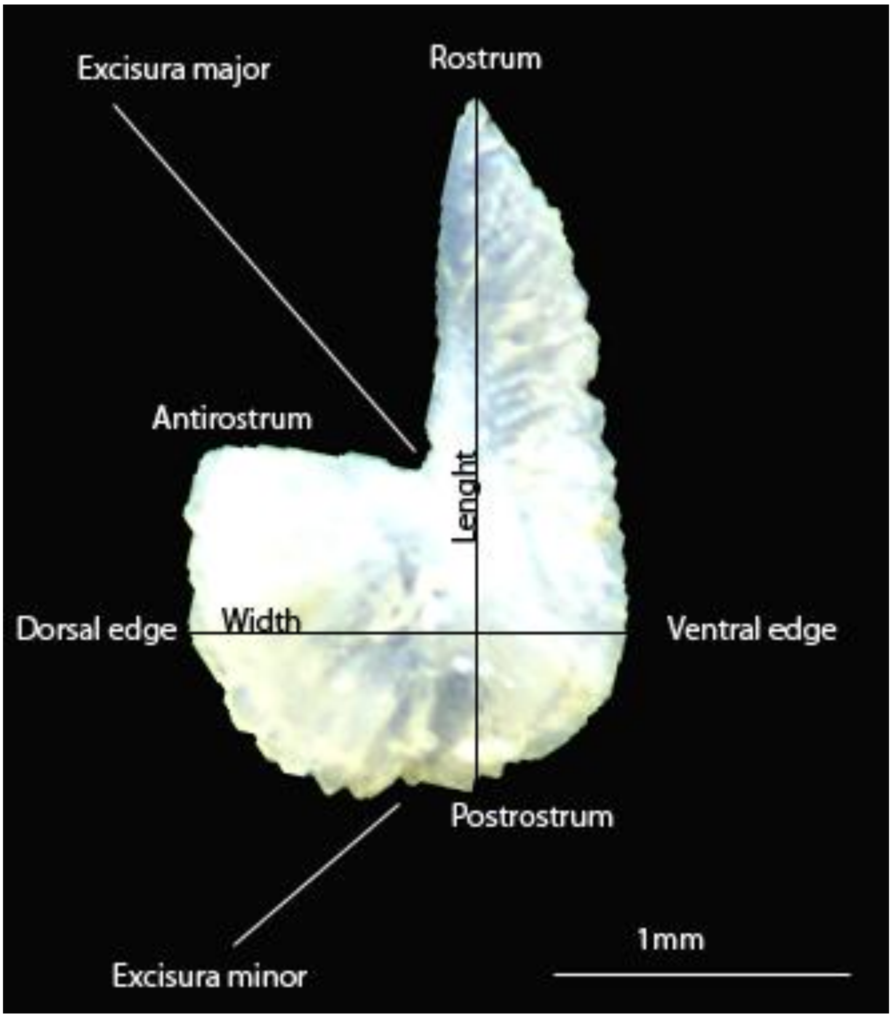

Figure 3: Mean otolith outline shapes formed with reverse Fourier transform of the outline using the first 39 harmonics, showing the overlap and variations between right (dotted line) and left (solid line) otolith shape of swordfish from the Indian Ocean (*n* = 91)

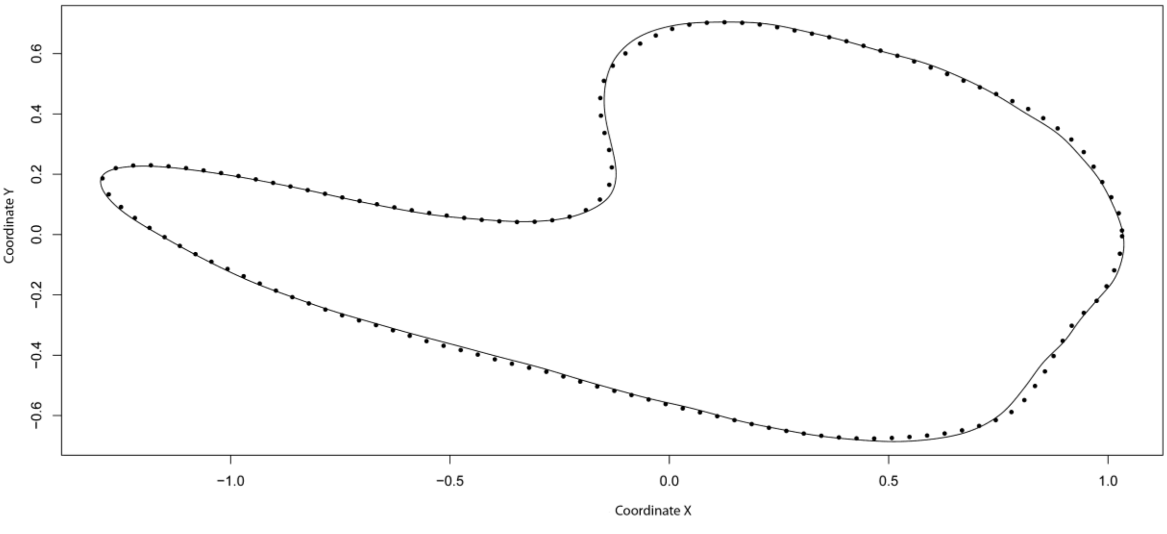

Figure 4: Average individuals (i.e. average otolith shape for each group of individuals) from the three clusters identified by hierarchical cluster analysis using Ward's hierarchical algorithm based on the squared Euclidean distances for all swordfish (n = 391) on the first two dimensions. The dots with sample numbers represent individuals

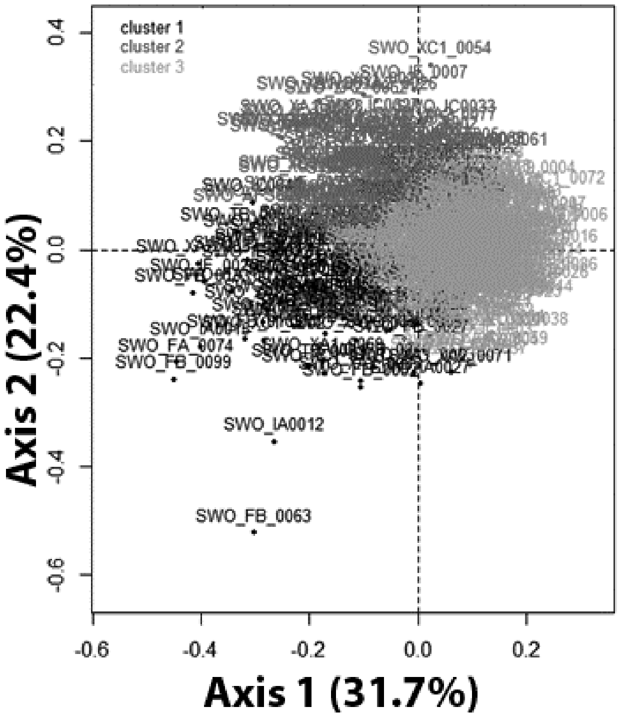

Table 1: Number of swordfish otolith samples by year, sex (not available for gutted fish), season (S1 = April–July; S2 = September–January) and sampling area. LJF = mean lower jaw fork length

Sampling area	LJF (cm)	Sex			Sampling year								Total
		Male	Female	Unidentified	2009		2010		2013		2014		
					S1	S2	S1	S2	S1	S2	S1	S2	
La Réunion	143.0±23.0	38	59	10	12	49	1	45					107
Mozambique Channel	135.1±38.6	11	10	13			34						34
Rodrigues	165.2±29.8	29	54	0		39		44					83
South Africa	185.9±36.9			20						5		15	20
Madagascar South	150.4±34.2	21	48	2	19	43	9						71
Sri Lanka	167.3±57.5	8	20	48		23	48	5					76
Total	155.0±38.6	107	191	93	31	154	92	94		5		15	391

Table 2: Jackknifed correct classification matrix of the LDA for mature swordfish (*n* = 391) between sampling areas based on the selected PC matrix. The percentages in each row represent the classification into the sampling area in each column (the correct classification is in the shaded squares on the diagonal). Overall classification success: 30%

Sampling area (*n*)	South Africa	Madagascar South	Mozambique	La Réunion	Rodrigues	Sri Lanka	%
South Africa (20)	4	3	2	1	9	1	20
Madagascar South (71)	5	20	4	20	9	13	28
Mozambique Channel (34)	3	2	5	11	9	4	15
La Réunion (107)	5	17	16	42	14	13	39
Rodrigues (83)	2	12	8	23	28	10	34
Sri Lanka (76)	2	12	14	13	12	23	30

Table 3: Classification matrix of the hierarchical clustering on principal components for mature swordfish (*n* = 391) between sampling areas, based on the selected PC matrix

Sampling area	Cluster 1	Cluster 2	Cluster 3	Total
South Africa	2	5	13	20
Madagascar South	11	20	40	71
Mozambique	3	13	18	34
La Réunion	20	23	64	107
Rodrigues	12	23	48	83
Sri Lanka	17	17	42	76
Total	65	101	225	391