Otolith microchemistry suggests probable population structuring in the Indian Ocean for the broadbill swordfish *Xiphias gladius*

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Abstract

Variation in otolith elemental fingerprints was investigated in the broadbill swordfish (*Xiphias gladius*) to complement genetic data obtained by next generation sequencing in the framework of a collaborative project on population stock structure of tuna, billfish and sharks of the Indian Ocean (PSTBS-IO). Swordfish specimens for this work were sampled in the southwest (SWI), west central (WCI) and southeast (SEI) regions of the Indian Ocean. A total of 70 otoliths (30 from SWI and 20 from each WCI and SEI) were selected and the elemental signatures of their cores were analysed by LA-ICP-MS to investigate potential differences in spawning origin among regions. Among the 15 chemical elements analysed, only Mg, P, Sr, Ba and B were above detection limits and significantly contributed to the variation in otolith core composition. Based on differences in these five elements, three groups of distinct multi-elemental signatures, denoting potentially discrete spawning origins (SpO), were identified using hierarchical clustering based on Euclidian distances. All SpO identified apparently contributed to the swordfish stocks of the three regions sampled, but in different proportions. SpO-1 was the most common spawning source among the fish sampled (49%); it probably corresponds to the swordfish spawning ground located between northeast Australia and Indonesia. SpO-3 was found to provide 34% of the total fish analysed, but mainly in SWI (53%) and WCI (35%). It could correspond to the spawning grounds reported for the species in the central and southwestern Indian Ocean. Lastly, SpO-2, which contributed to only 17% of the total fish analysed (mainly in SEI and WCI), may correspond to the spawning ground previously reported in the northwestern Indian Ocean, off the Somalian coast. Although our results show mixed origins in the fish sampled at each sampling location, the contrast in otolith core fingerprints between SWI and SEI otoliths suggests differences in main spawning origin, at least for the swordfish captured in these two regions of the Indian Ocean. Additional analyses are needed to consolidate these results, as well as information on the spatiotemporal distribution of chemical tracers in the water masses of the Indian Ocean to assign regions to otolith elemental signatures.

1- Introduction

Management of fish resources requires knowledge on the connectivity patterns responsible for the genetic structure of exploited stocks, especially for species with a life cycle that involves migration at varied life stages (Costello et al. 2010). Yet, this information is still lacking for many target species and parts of the world. Pelagic predators such as tunas, swordfishes and sharks often undertake large oceanic migrations at different stages of their life, which makes the sustainable management of these species a complex and formidable challenge (Rooker et al., 2008; Block et al., 2011). The swordfish *Xiphias gladius* is a key species in commercial fisheries, but management efforts continue to be hindered by data gaps in its basic biology, including ongoing debate over stock boundaries and movement between management zones (Braun et al. 2019; Erauskin-Extramiana et al. 2020). Because the species is known to migrate across entire ocean basins (Neilson et al., 2009; Abascal et al., 2015) improved understanding of its population structure and identification of its key habitats are urgently needed. In particular, stock structure of this species remains hypothetical in the Indian Ocean (West, 2016), and few electronic tagging data to inform movements have been collected in the recent years (West at al., 2012; Romanov et al., 2016).

Over the last decades, otolith microchemistry has emerged as a very powerful tool for assessing lifetime migrations and population structure in marine fish (Sturrock et al. 2012), mainly because it is capable of gathering reliable information even from fish early life stages, which are often the most difficult to capture and track at sea (Hazen et al. 2012). Otoliths are small calcified structures located in the inner ear of the fish. They are regularly referred to as fish 'black boxes' as they may provide a time resolved and temporally stable record of the environmental conditions experienced by individual fish throughout their life (Campana 1999). Indeed, otolith chemical composition reflects, at least partly, the physicochemical conditions in the water surrounding the fish at the time of deposition (Elsdon & Gillanders, 2003; Elsdon et al., 2008). Because otoliths are acellular and metabolically inert, their material is neither reabsorbed nor reworked after deposition (Panfili et al., 2002). Therefore, their chemical signatures provide fingerprints of the water masses successively inhabited by the fish, acting as natural tags (Campana et al., 2000; Elsdon & Gillanders, 2003; Darnaude & Hunter, 2017) that can be used to reconstruct the environmental history of sampled individuals or identify migratory and life-history patterns within populations (Walther et al. 2017). So far, otoliths have been successfully used to identify natal origins, population structure and movements of varied large pelagic fish (e.g Artetxe-Arrate et al., 2019, Fraile et al., 2016; Rooker et al., 2016, Baumann et al., 2015). In the present work, we took advantage of this valuable source of information to explore potential variation in spawning origin in broadbill swordfish (*Xiphias gladius*) from different regions in the Indian Ocean. Analyses of the multi-elemental composition of the otolith central area, which reflect the environmental conditions experienced by the fish during the very first months of their lives, were conducted to complement genetical analyses and help delineate swordfish stocks. This study is part of the recently completed "Population Structure of IOTC species and sharks of interest in the Indian Ocean" (PSTBS-IO, 2017-2020). Prior to this study, investigation of otolith microchemistry of *X. gladius* has been limited to the Pacific Ocean: solution-based ICP-MS was used to obtain otolith elemental fingerprints from varied sites around the Hawaiian Islands and demonstrated the existence of distinct swordfish nursery areas in this region (Humphreys et al., 2005). In the Indian Ocean, only otolith shape has been analysed for the species (Mahe et al., 2016). No significant differences in shape were found among the otoliths of adult individuals collected at six locations in the western and central Indian Ocean. However, given the multiplicity of factors (environmental, ontogenetic, sexual and genetic) that might influence otolith shape (Mille et al., 2016; Mahé et al., 2019) and bias its use for stock delineation, the microchemical data gathered in the present work, although preliminary, could provide valuable new information to complement data on swordfish genetic differentiation within the Indian Ocean.

2- Material and Methods

2.1 Sampling

Due to sampling limitations during the project, swordfish otoliths were collected in three regions only, the southwest Indian Ocean (SWI; n=33), the west central Indian Ocean (WCI; n=53) and the southeast Indian Ocean (SEI; n=38). For SWI and WCI, samples were collected from the domestic longline fleets based in La Réunion and Seychelles, respectively. SEI samples were collected from the domestic longline fleet based in Fremantle, Australia. Microchemical analyses were performed only on the otoliths of a selection of 70 of these individuals, all with intact left otoliths (sagittae): 30 from the SWI, 20 from the WCI, and 20 from SEI (Figure 1). These fish ranged from 80 to 226 cm in size (lower jaw fork length, LJFL). They were considered either sub-adults or adults, as the estimated LJFL at 50% maturity is 120 cm for males and 170 cm for females (Poisson and Fauvel, 2009), and their probable ages ranged from one to 10 years (Table 1). Therefore, only their final sampling origin (region of capture) was considered when interpreting results from otoliths analyses.

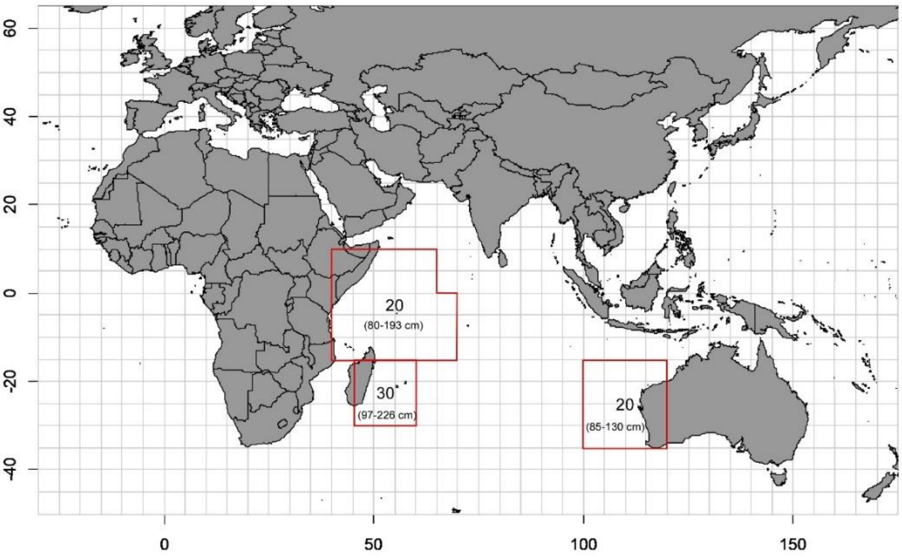

Figure 1. Number of swordfish otoliths analysed and size range of individuals (in brackets) for each of the three sampling locations, referred to as southwest Indian Ocean (SWI), west central Indian Ocean (WCI) and southeast Indian Ocean (SEI).

Table 1. Number, sampling period, size range and estimated ages of fish for each of the three sampling locations SWI, WCI and SEI.

Location	**Number analysed**	**Sampling dates**	**LJFL (cm)**	*** Estimated age range (years)**
Southwest Indian Ocean (SWI)	20	Nov-Dec 2017	97-226	1-10
Southwest Indian Ocean (SWI)	10	Dec 2018	111-204	2-8
West central Indian Ocean (WCI)	20	Mar-May 2018	80-193	1-7
Southeast Indian Ocean (SEI)	20	Mar-May 2018	85-130	1-3

* The ranges in ages are for male and females combined (Varghese et al. 2013, Wang et al. 2010).

2.2 Otolith preparation

All materials for otolith handling, preparation and analysis were decontaminated in 4% ultrapure nitric acid baths, rinsed with ultrapure (18.2 MQ) water and dried under a Class 100 laminar flow hood. For each individual fish, the otolith was sonicated for 5 min in ultrapure water and dried under the same laminar flow hood. It was then embedded in epoxy resin (Araldite 2020) polymerized in an oven at 35 °C for 24 h and transversal sections (of 1 mm on average) including the nucleus were made using a precision saw (Bluehler®, Isomet 1000). The posterior face of the otolith sections were then polished using 1200, 2400 and 4000 grit dry abrasive papers until the core was reached. The sections were then sonicated for 5 min in ultrapure water, dried under a class 100 laminar flow hood and attached to a clean microscope slide for further processing.

2.3 Trace element analyses

Element concentrations in the otoliths were measured using Laser Ablation ICP-MS (FINNIGAN-Element2 XR coupled to a Lambda Physik 193 nm laser) at the AETE-OSU OREME facility of the Montpellier University (France). For each otolith, the concentrations were measured along a transect from the core to the edge. A pre-ablation transect was used to clean the otolith surface (pulse rate 4 Hz, energy 15 J cm^{-2} and spot diameter 80 µm), and then the ablation transect was analyzed for measuring the chosen elements (pulse rate 7 Hz, energy 15 J cm^{-2} and spot diameter 50 µm). For calibration and quality control, a glass reference material (NIST 612 - National Institute of Standard and Technology, USA) was analysed at the beginning, after every 5 samples and at the end of each session. Another reference material (MACS 3) was analysed at the beginning and at the end of each session (Ladislav et al. 2009; Rodriguez et al. 2008). To remove residual sample gas that could interfere with the analysis, the laser chamber was purged for 30 seconds before analysing each sample. Fifteen chemical elements were measured. Calcium was used as the internal otolith standard, and results were given as ratios to Ca. All raw data were processed using the elementR package for R (Sirot et al. 2017). This procedure corrected the machine drift using the reference materials, filtered the levels of detection for each element, discarded blanks at the beginning and end of the analysis, and then normalized to Ca^{43}. After examination of the levels of detection for the 15 elements analysed by ICPMS, 6 elements were retained for further analysis (B, Mg, P, Zn, Sr and Ba).

Elemental ratios in the otolith core can be affected by egg yolk composition (Brophy 2004, Ruttenberg et al. 2005, Mcdonald et al 2008). Consequently, the value centred on the otolith primordium was removed from our analyses, in order to avoid any maternal influence on otolith composition. The mean of the next three points, between 10 and 40 microns after the core (or 'near-core signature') was chosen to reflect fish spawning origin(s), i.e. the characteristics of the water mass(es) encountered during the first weeks of larval drift at sea.

2.4 Statistical analyses

Differences in natal origins were investigated using ‘near-core signatures’ in B, Mg, P, Zn, Sr and Ba. For this, a Principal Component Analysis (PCA) was first made using the R package FactoMineR to identify the main elemental ratios responsible for data separation. We then used agglomerative Ward's hierarchal clustering on the concentrations of the elements identified by PCA to investigate the number of fish spawning origins, i.e. find groups of individuals that had shared similar otolith signatures during their early larval life. For this, the number of potential spawning origins (clusters) was investigated using the R package NbClust (Charrad et al. 2014) which identifies the most frequent number of clusters (k) proposed by 30 different clustering indices. Here, following Murtagh and Legendre (2014), the procedure was performed with the ‘ward.D2’ methods to preserve the Ward’s clustering criterion and by computing Euclidian distances on data formerly centred and reduced, as this clustering technique tends to give more weight to large clusters and is less likely to identify small larval sources.

A combination of univariate and multivariate statistical techniques was used to look at the differences in single and multi-element concentrations of otolith near-core areas according to fish spawning cluster (k potential spawning origins) or final capture region (SWI, WCI or SEI). As the assumptions for normality and homoscedasticity were not met for all six elements, a PERMANOVA test was first performed, followed, when relevant, by separate Kruskal Wallis tests and Pairwise Wilcoxon tests for each element. All statistical analyses were performed using the R software version 3.6.0 (R development Core Team 2019) taking $\alpha < 0.05$ as the threshold for statistical significance. Data normality and homoscedasticity were checked using Shapiro-Wilk and Box M tests, respectively.

3- Results

The near-core otolith signatures of the swordfish from three sampling regions largely overlapped (Figure 2). However, slight but significant differences in otolith natal fingerprints were observed according to the fish final capture site (PERMANOVA, $p = 0.047$). In particular, near-core concentrations in B, Ba, Mg, and P were significantly higher ($p<0.02$) for the fish captured in the SEI than for those caught in the SWI (Figure 2). Swordfish captured in the WCI exhibited intermediate near-core signatures, which only differed from those of the fish captured in the SWI and in the SEI in terms of Mg ($p< 0.001$) or in terms of Ba and P ($p< 0.05$), respectively.

Among the six elements successfully measured in the otoliths, only Mg, P, Sr, Ba and B significantly contributed to interindividual variation in near-core signatures (>20% for axes 1 and/or 2 in the PCA; Figure 2). Therefore, only these five elements were retained for further investigation of fish spawning origin. Based on the interindividual variation in the elemental concentrations, the most likely number of clusters (i.e. of distinct spawning origins, SpO) among the swordfish tested was found to be three (Figure 3). These three SpO had significantly distinct multi-elemental signatures (PERMANOVA, $p = 0.002$; Figure 4). In particular, B differed significantly among all SpO, with maximum values in SpO-1 and minimum ones in SpO-3. SpO-2 had significantly higher Sr and Ba signatures and SpO-3 had lower Mg and P signatures than the two other SpO (Figure 5).

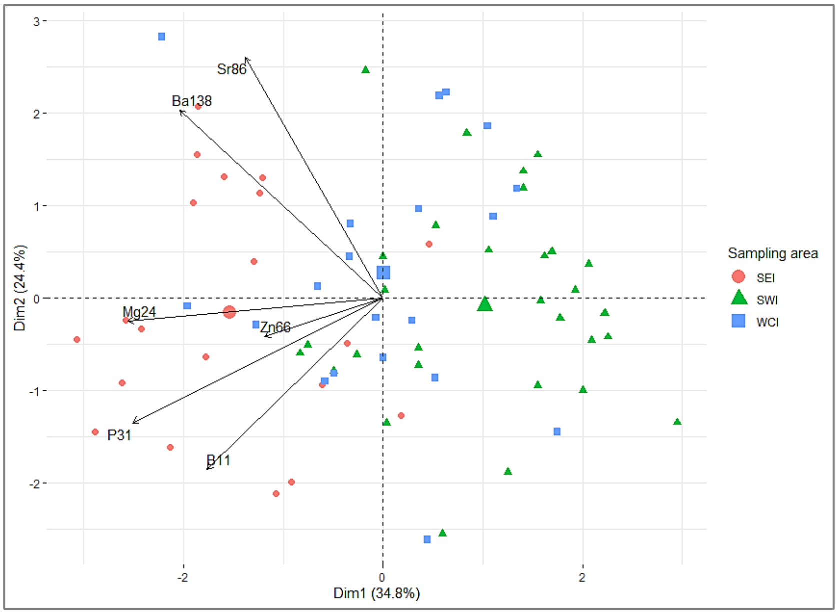

Figure 2 – Biplot of individual (fish) and variable (chemical elements) projection on the first plane of the PCA (49.2%) made with the swordfish otolith near-core signatures. Individuals are coded by their sampling region (SEI, SWI or WCI). For the variables, the length of the arrow reflects the % of contribution to the total inertia.

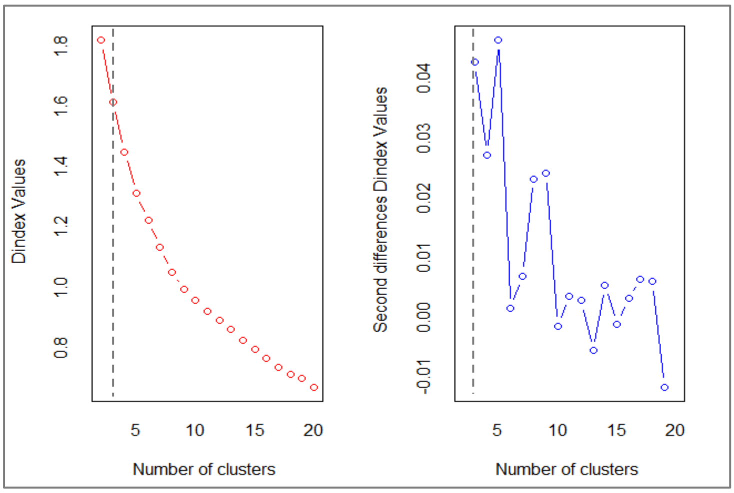

Figure 3. Results of the NbClust function (Charrad et al. 2014) using hierarchical clustering with the Ward method and Euclidian distances. On both graphs, the dotted line shows the most likely number of clusters defined according to the majority rule among all indices (here k =3).

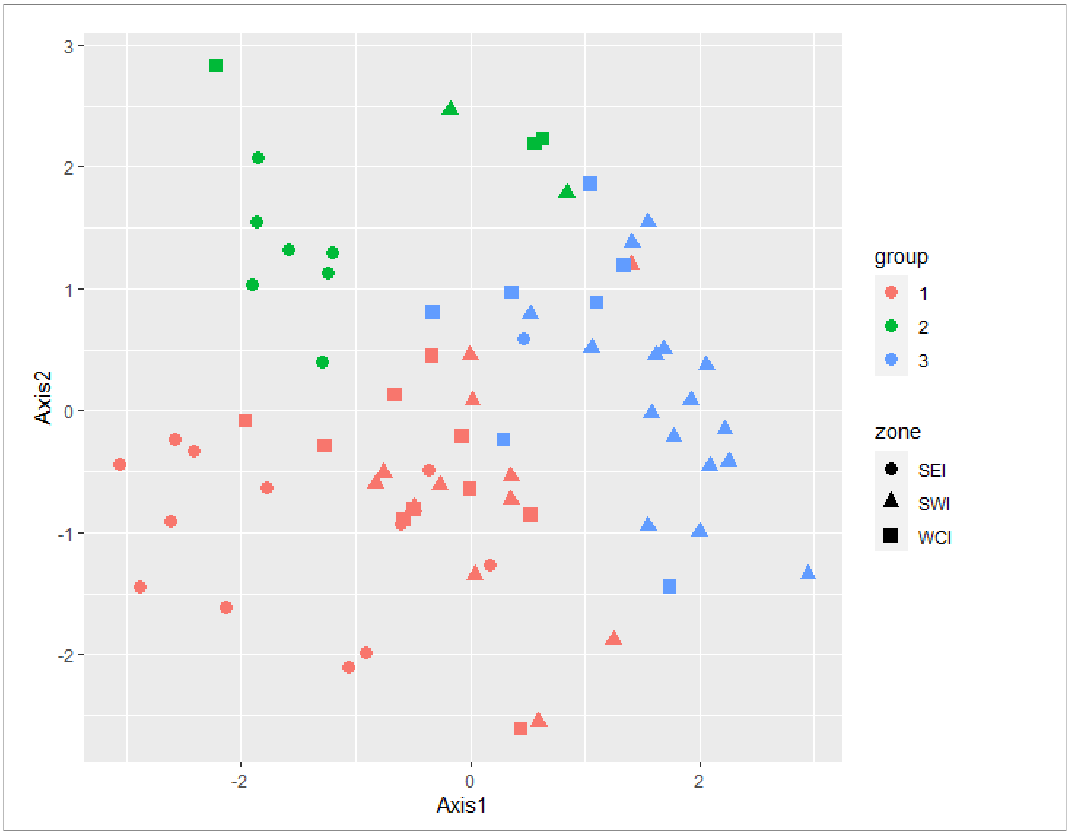

Figure 4. Projection of the clusters identified as potential discrete spawning origins (SpO 1 to 3) on the first plane of the PCA made with the near-core multi-elemental (B, Mg, P, Sr, Ba) signatures of the otolith of the 70 swordfish analysed. Colours on the graph represent the spawning origin (1 to 3) for each fish and symbols represent the region of capture.

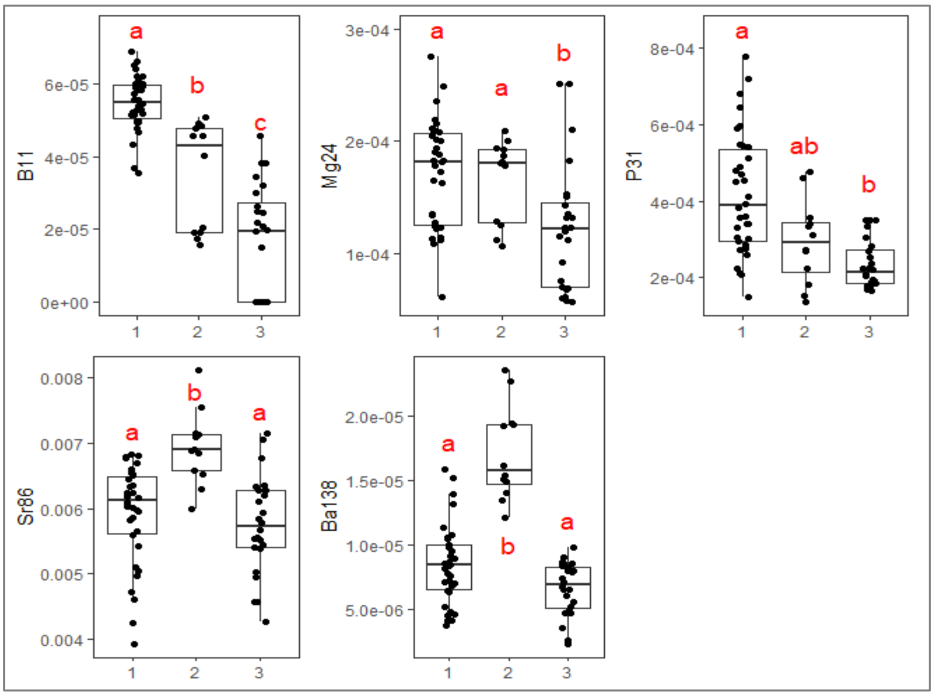

Figure 5. Near-core elemental signatures (black dots) of the 70 swordfish analysed and corresponding boxplots (Q1, median, Q3). Letters in red indicate groups with significantly distinct signatures ($p<0.05$).

All three SpO apparently contributed to the stocks exploited in each of the three regions investigated, however in different proportions (Figure 4). SpO-1 was the main spawning source for the individuals sampled (49%). By region, it provided 40-60% of the fish analysed (Table 2). SpO-3 provided 34% of the fish sampled, principally in SWI (53%) and WCI (35%). Its contribution to SEI was very low (5%). SpO-2 contributed to only 17% of the total number of fish analysed but its contribution was 2 to 5 times greater in SEI (35%) compared to WCI (15%) and SWI (7%). Differences in spawning origin among sampling regions explained the differences in near-core signatures observed between the swordfish from SEI and SWI in the original PCA (Figure 2), as the former mainly (95%) originated from SpO-1 or SpO-2 when more than half of the later (53%) came from SpO-3.

Table 2. Relative proportion (%) of sub-adult and adult swordfish individuals from each spawning origin (SpO 1 to 3) analysed (total number of fish tested = 70, LJFL = 80-226 cm), for each of the three sampling regions in the Indian Ocean: Southeast (SEI), Southwest (SWI) and West Central (WCI).

	SEI	SWI	WCI
SpO-1	60%	40%	50%
SpO-2	35%	7%	15%
SpO-3	5%	53%	35%
Total	**100%**	**100%**	**100%**

4- Discussion

Studies examining nuclear DNA microsatellite markers so far have failed to demonstrate evidence of population structure for swordfish in the Indian ocean (Muths et al., 2009; Muths et al., 2013). However, heterogeneity of Indian Ocean swordfish has been detected through examination of maternally transmitted mtDNA markers (e.g. Lu et al., 2006; Bradman et al., 2011), and the analysis of SNP genotypes recently suggested the presence of subtle population structure for the species in this area (Grewe et al., 2020). Our results further confirm this possibility. Indeed, multi-elemental signatures of otolith cores indicate three potentially distinct spawning origins in the 70 sub-adult and adult swordfish (*X. gladius*) captured between November 2017 and December 2018 in the southeast, southwest and west central regions of the Indian Ocean. All three putative spawning origins apparently contribute to each of the swordfish aggregations fished in the three regions sampled, but in varying proportions.

For *X. gladius*, breeding females have been reported in the second and the third quarters of the year in the northwestern Indian Ocean and in the first and the fourth quarters in the area surrounding Reunion Island (Poisson and Fauvel, 2009). This means that reproduction in the species occurs under similar conditions of SST and seasonal light regimes (spring and summer) but at different moments of the year in northern and southern hemispheres. These differences complicate spawning site geolocation using otolith microchemistry. Indeed, seasonality can strongly influence otolith signatures, even at sea (Thomas et al., 2020), and should be considered in any study aiming to retrospectively locate fish. Ideally, young-of-the-year fish would have been sampled from each location in the same year to minimize temporal variability in otolith signatures. Limitations in sample collection did not allow this scheme to be achieved in the present work. The size-range of the fish analysed was 80 to 226 cm LJFL and with such a large range in size (and age), the results must be interpreted with caution. Indeed, differences in otolith core signatures might be due in part to cohort effects, i.e. they might be the result of inter-annual or seasonal shifts in oceanic water composition at one location rather than swordfish otoliths having different geographical origins. This being said, otolith elemental signatures can provide useful information on the physicochemical characteristics of the water masses in which spawning occurred for each individual. For example, barium (Ba) and strontium (Sr) incorporation into otoliths has been shown to be correlated with ambient water concentrations (Webb et al. 2012; Izzo et al. 2018). Although both elements have been related to salinity (e.g. Elsdon and Gillanders, 2005; Macdonald and Crook, 2010), Ba variations do not solely reflect changes in salinity. Indeed, because dissolved Ba in seawater possesses a nutrient-like profile (i.e. very low concentration in surface waters and increased values in deeper ones), increases in otolith Ba concentrations may reflect periods of residence in upwelling zones (Patterson et al., 2004; Kingsford et al., 2009; Wang et al. 2009). The summer monsoon upwelling off the Somalian coast (Wiggert et al. 2006) could therefore also cause high Ba concentrations in the otolith of swordfish born in this area of the northern hemisphere. Uptake rates of boron (B), magnesium (Mg) and phosphorus (P) into otoliths are more complex, but otolith concentrations in these elements often reflect their availability in the surrounding water, at least in part (Sturrock et al. 2012). Based on the available knowledge on swordfish spawning grounds (Nishikawa et al., 1974; 1985; García-Cortés and Mejuto, 2003; Poisson and Fauvel, 2009), surface circulation patterns (Schott and McCreary, 2001) and water composition in various areas of the Indian Ocean (e.g. Rochford, 1962; Wiggert et al., 2006; 2013; Krishna et al., 2016), we propose preliminary hypotheses on the geographical location of the three spawning origins identified here:

- **SpO-1**, characterized by high B, Mg and P signatures, but low Sr and Ba values, appears to be the most common spawning source in the Indian Ocean (49% of swordfish otolith cores had its elemental signature). Actually, SpO-1 signature was the most common in each of the three sampling regions, with a minimum of 40% in SWI and a maximum of 60% in SEI. This suggests that SpO-1 may correspond to the swordfish spawning ground (indicated by high larval density, Nishikawa et al., 1974; 1985) located between Northeast Australia and Indonesia;

- **SpO-2** contributed to only 17% of the total number of fish analysed (35% in SEI, 15% WCI and 7% SWI) but is characterized by high Sr and Ba signatures. It may therefore correspond to the spawning ground in the Western Indian Ocean described by Garcia-Cortés and Mejuto (2003) in their IND51 area (10°N-5°S / 40°-55°E), which is under the influence of the Somalian upwelling in the summer;

- **SpO-3**, characterized by low signatures in all five elements, provided 34% of the total fish analysed, mosty in in SWI (53%) and WCI (35%). Therefore, this spawning origin could gather the swordfish spawning sites reported in the central and southwestern parts of the Indian Ocean, including those near the Reunion Island (Poisson and Fauvel 2009).

In light of the seasonal and interannual variability in environmental conditions that exist within the Indian Ocean (Schott and McCreary, 2001), these preliminary results will need to be confirmed by the analysis of a larger number of samples. Ideally, otolith fingerprints would need to be assessed for young-of-the-year from the same cohorts, collected in several successive years (at least three) from the three regions investigated here. In addition, we suggest collecting swordfish from other locations in the Indian Ocean where juveniles are caught, where adults are known to spawn or where larvae have been reported. These samples could then be used to test whether the hypothetical spawning ground clusters found in this study correspond to spatially distinct spawning zones. Additionally, an investigation on the distribution of biogeochemical tracers of the Indian Ocean water masses (through literature and operations) would provide highly valuable information for the interpretation of the elemental composition of otoliths (core and edge). In combination with genetic data, the information from otolith microchemistry would then give a clear pattern of the population structure of the species in the Indian Ocean.

Acknowledgements: This work was funded by the European Union and the consortium partners, under the IOTC, in the frame of the recently completed “Population Structure of IOTC species and sharks of interest in the Indian Ocean” (PSTBS-IO, 2017-2020). The project was conducted by an international consortium led by the CSIRO (Australia) and including the IRD and the Ifremer (France), the AZTI (Spain) and the RITF (Indonesia). We would like to thank O. Bruguier (Montpellier University) for its contribution to LA-ICPMS analyses.

Nishikawa et al., 1974; 1985

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