Received: 11 October 2013

IOTC–2013–WPTT15–36

DISCRIMINATION OF YELLOWFIN TUNA FROM THE PUTATIVE NURSERIES OF THE WESTERN INDIAN OCEAN

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ABSTRACT

Stable carbon and oxygen isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) were measured in otoliths of young of the year yellowfin tuna (*Thunnus albacares*) collected from different nursery areas in the western Indian Ocean. Samples were obtained from February 2009 to May 2010 from three regions that include a variety of physical characteristics and habitat types: Somali waters in the northwestern Indian Ocean (0-10ºN), surrounding waters of the Seychelles Islands (0-10ºS) and northern Mozambique Channel (13-16ºS). Somalia and Seychelles region did no show a significant difference in otolith isotopic signature and thus, fish collected in these regions were pooled together and compared with those collected in Mozambique Channel. Significant differences existed in $\delta^{18}O$ values among the two nurseries, with more depleted values in fish collected in Mozambique Channel compared with those collected in Seychelles-Somalia region. Cross-validated classification success, based on quadratic discriminant analysis, was relatively high, with 70% of the fish correctly classified to their respective nursery areas. The ability to discriminate individuals from the putative nurseries in the western Indian Ocean allows estimating the degree of connectivity and natal homing of adult individuals. In this way, otolith $\delta^{13}C$ and $\delta^{18}O$ of young of the year yellowfin tuna were used as a baseline to predict the origin of juvenile and adolescent individuals captured in Somali waters. Our results reveal that about 80% of yellowfin tuna captured in Somalia region were originated from the same nursery, highlighting the importance of local production. We believed that these comparisons will provide useful information on which nursery areas are the most important for the yellowfin tuna fishery in the Indian Ocean.

KEYWORDS

Yellowfin tuna, Indian Ocean, otolith, microchemistry, mixing.

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Introduction

Yellowfin tuna (*Thunnus albacares*) is a large epipelagic species widely distributed in the tropical and subtropical waters of the major oceans (Collette and Nauen, 1983). It is an important component of tuna fisheries worldwide and is one of the major target species for the tuna fishery in the Indian Ocean (Somvanshi, 2002). Total annual catch of yellowfin tuna in the Indian Ocean has increased significantly since the beginning of industrial fishing in the early 1980s, with the advent of the purse seine fishery. Last stock assessment results suggest that that the stock is currently not overfished and overfishing is not occurring and, thus, IOTC recommended that catches of yellowfin tuna should not exceed the estimated Maximum Sustainable Yield of 300,000 tonnes per year (IOTC, 2012). The assessment of the yellowfin tuna population in the Indian Ocean is based on the assumption that it constitutes a single stock. However, there is not sufficient information to confirm accurately this hypothesis, and several studies suggest that the population and spatial dynamics could be more complex in the Indian Ocean (Dammannagoda et al., 2008; Chow et al., 2000; Kunal et al., 2013).

Yellowfin tuna is a batch spawner with an indeterminate fecundity (Zudaire et al. 2013a,b). This species has a spawning frequency of about 1.5 days (McPherson, 1991; Schaefer, 2001), with two main spawning periods described in the Indian Ocean: first period from November to March and a second period in June (Zudaire et al., 2013b). Temperature (>24ºC) seems to regulate spawning activity of yellowfin tuna (Schaefer, 2001), who disperse the eggs over a vast area of the tropical zone throughout the year (Stéquert et al., 2001; Zudaire et al., 2010). Although is one of the most important resources of the Indian Ocean fisheries, fundamental questions remain regarding the location of spawning areas, connectivity between larval pools and the spatial dynamics of subpopulations.

Among the recent advances for the assessment of fish stock structure, otolith microchemistry has emerged as an efficient tool to identify nursery habitats and migration paths (Campana et al., 1994). The technique relies on the basic assumption that as the otolith grows throughout the lifetime of the fish by precipitation of calcium carbonate layers, chemical markers from the ambient environment are incorporated into its microstructure, resulting in a fingerprint that reflects the physicochemical properties of the environment in which it was formed (Campana et al., 1994, Bath et al., 2000). Thus, material deposited in the core of the otolith during the first weeks or months of their life may serve as a marker of an individual's natal origin, allowing the identification of adult fish to their corresponding nursery area by association between chemical composition of the otolith core and water chemistry. Carbon and oxygen isotope ratios ($\delta^{13}C$ and $\delta^{18}O$) in otoliths have been previously used to determine the natal origin of tuna

species in the Atlantic and Pacific Oceans (Rooker et al. 2008a,b, Schloesser et al. 2010, Wells et al. 2012). In the Indian Ocean, spawning occurs mainly in the western equatorial area (IOTC 2012), but the degree of connectivity between the different nursery areas remains unknown. A better understanding of the population structure of yellowfin tuna in the Indian Ocean is necessary to improve stock assessment and management of the resource.

Here we use otolith $\delta^{13}C$ and $\delta^{18}O$ isotopes to discriminate among different nursery areas of the western Indian Ocean. Otoliths of young of the year (YOY) yellowfin tuna collected in different regions of the western Indian Ocean were analyzed to determine whether yellowfin tuna from these regions could be discriminated based on their isotopic composition. We tested the usefulness of three distinct classification methods: Quadratic Discriminant Analysis (QDA), Random Forest (RF) and Artificial Neural Networks (ANN), and found that QDA provides the overall best results for discrimination of the YOY yellowfin tuna to their respective nurseries. Using YOY otoliths as a baseline sample set, juvenile and adolescent individuals collected in the northwestern Indian Ocean were then compared with the reference samples and their natal origin was predicted using QDA.

Material and Methods

Fish collection

YOY yellowfin tuna otoliths were collected from the nursery areas of the western Indian Ocean: (1) Somalia region (0-10ºN); (2) Seychelles (0-10ºS) and (3) Mozambique Channel (13ºS-16ºS) (Fig. 1). Yellowfin tuna are believed to be broadcast spawners, thus, nurseries were defined as regions of the western Indian Ocean inhabited by YOY yellowfin tuna. Yellowfin tuna are fished throughout the Indian Ocean, with the majority of the catches being taken in the western equatorial waters. Samples were collected using the Spanish purse seiners fleet operating in the Indian Ocean between February 2009 and May 2010 (Table 1). YOY tuna obtained of the pelagic purse seiner fishery targeting adult yellowfin tuna (*Thunnus albacares*), skipjack tuna (*Katsuwonus pelamis*) and bigeye tuna (*Thunnus obesus*) were used in the present study. Fish size ranged between 29 and 43cm fork length (FL), with a mean size of 35cm FL. Additionally, Age-1 and Age-2 yellowfin tuna (n=5) were collected during February 2009 in Somali waters. Otoliths of these juvenile and adolescent yellowfin tuna were compared with the YOY yellowfin tuna used as reference samples.

Otolith preparation and analysis

A total of 23 pairs of sagittal otoliths were removed from the specimens, cleaned of biological residue and stored dry in plastic vials. In the laboratory, otoliths were soaked briefly in dilute nitric acid (1%) to eliminate any remaining biological material and then rinsed with deionised water. One otolith per fish was randomly selected for carbon and oxygen isotope analysis. Samples were prepared for chemical analysis as described by Rooker et al. (2008b). Otoliths were embedded with Epofix resin (Stuers), and transverse sections of approximately 1.5mm thick were cut across the core using an isomet low-speed saw. Sections were glued in a sample plate with thermoplastic glue. Microsampling of otolith powder for carbon and oxygen isotope analysis was performed using a high resolution compuretized micromill (New Wave MicroMill System) consisting in a microscope and imaging system, controlled by a computer software. The portion of the smallest YOY fish was used to create a standard template that was then used with the remaining otoliths, to ensure that exactly the same portion of the otolith is analyzed in every fish (approximately the first 4 mo of life according to Dortel et al., 2013). Aproximately 14 drill passes were run at 50 µm depth per pass over a pre-programmed drill path using a 200 µm diameter carbide bit (Komet dental, Gebr. Basseler, GmbH & Co, KG). Powdered material was collected in a weighing paper and stored in plastic vials until permorming the analysis.

Carbon and oxygen stable isotopes of otolith samples were analysed on an automated carbonate preparation device (KIEL-III) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252) from the Environmental Isotope Laboratory of the University of Arizona. All isotope values are reported according to standards of the International Atomic Energy Agency in Vienna. $\delta^{13}C$ and $\delta^{18}O$ represent the ratio between the $^{13/12}C$ and $^{18/16}O$ in the sample, stated in per mil, relative to the Pee Dee Belemnite (PDB) scale.

Statistical analysis

All isotope values were standardised before any analyses to minimize the heterogeneity of variance across regions. We explored variation in $\delta^{13}C$ and $\delta^{18}O$ of YOY yellowfin tuna capture locations using multivariate analysis of variance (ANOVA and MANOVA). Pillai's trace was selected as the multivariate test statistic since it is relatively robust to deviations from multivariate normality (Johnson and Field, 1993). We evaluate the ability of three classification methods to separate individual YOY yellowfin tuna to their respective nurseries: Quadratic Discriminant Analysis (QDA) was compared with two machine

learning methods such as Random Forest (RF) and Artificial Neural Networks (ANN), less demanding in terms of assumptions than classical QDA. The training set was comprised by the 75% of the values, and the remaining 25% of the data were used to determine classification accuracy of each of the method. Cross-validation was used to determine if single elements isotopes or combination of both carbon and oxygen isotopes optimised classification accuracy for the QDA method (detailed information described in Mecier et al., 2011). To get reliable results, we measured prediction efficiency over 1000 replicates and calculated the mean accuracy. Additionally, QDA has been used for individual origin assignment. QDA generates the probability that an individual fish belongs to a source population using the estimated discriminant model. All statistical analyses were carried out using open access software R (R Development Core Team., 2008; http://www.r-project.org/).

Results and Discussion:

MANOVA indicated that differences in isotopic signatures of YOY yellowfin tuna otoliths captured in Somalia region and Seychelles Islands were not significant. Thus, otoliths collected in these two regions were combined and treated as a single group, representing yellowfin tuna captured in Somalia-Seychelles region. Differences in carbon and oxygen stable isotopes between otoliths of YOY yellowfin captured in Seychelles-Somalia waters and Mozambique Channel were then statistically analysed. Significant variation in $\delta^{13}C$ and $\delta^{18}O$ concentration from otoliths of YOY yellowfin tuna was observed between the two putative nurseries sampled (MANOVA, $p < 0.05$). Separation of the two regions was due to a significant enrichment of $\delta^{18}O$ in otoliths of YOY yellowfin tuna collected in the Somalia-Seychelles compared with those captured nearby Mozambique Channel (ANOVA, $p < 0.05$; Fig. 2). The warm and fresh surface waters from the Tropical Surface Water mass found in northern Mozambique Channel region, formed by surface warming and excess of precipitation, may be responsible for a reduced salinity of the region compared to northern Indian Ocean (New et al., 2007), and in consequence for a depletion of water $\delta^{18}O$ composition.

Three classification methods have been compared in order to choose which statistical method performs the best and has a higher accuracy in habitat discrimination. Prediction efficiency was relatively high for the three classification methods, although QDA had the best performance among the three methods, with 70% of the YOY yellowfin tuna correctly classified to their corresponding nursery areas (Table 2). The

incorporation of carbon isotope data did not improve prediction efficiency, thus $\delta^{18}O$ values displayed the greatest accuracy of classification. The two potential nurseries for yellowfin tuna throughout the western Indian Ocean, Seychelles-Somalia and Mozambique Channel, have a sufficiently distinct isotopic signal to discriminate individuals from different nursery areas. Thus, the baseline sample set built with carbon and oxygen isotope data from YOY yellowfin tuna captured in these locations may serve as a baseline sample to compare with otolith data of adult yellowfin tuna and predict their nursery origin. Based on a similar methodology, Wells et al. (2012) discriminated several nursery areas across the equatorial Pacific Ocean using $\delta^{13}C$ and $\delta^{18}O$ composition of young of the year yellowfin tuna, and found a high degree of local production within the Hawaiian Islands. In the same way, we aimed to determine if adult and subadult yellowfin tuna captured in the northwestern Indian Ocean were locally produced, or a high degree of mixing occurred between the two regions investigated. Otoliths of juvenile and adolescent yellowfin tuna (Age-1 and Age- 2) captured in Somali waters during the same cruise were analyzed and compared to YOY isotope signatures to predict the origin of these fish (Fig. 3). $\delta^{13}C$ of juvenile and adolescent yellowfin tuna ranged from -10.51 to-9.32, with an average value of -9.93, and $\delta^{18}O$ values were between -2.24 and -1.51, with an average value of -1.84. The majority of these individuals (4 out of 5) collected in Somali waters were classified by QDA as fish originated in Seychelles-Somalia region, at a probability > 99%, whereas the remaining individual had a 54% of probability to belong to the source population of Mozambique Channel.

Conclusions

Although our dataset is very limited, the results from otolith stable isotope analysis provide some preliminary insights into yellowfin tuna stock structure and connectivity between possible subpopulations in the western Indian Ocean.

The similarity in otolith $\delta^{13}C$ and $\delta^{18}O$ of YOY yellowfin tuna collected in Somali waters and around Seychelles Islands show little differences, suggesting some mixing between larval pools of these two regions. In contrast, otoliths of YOY yellowfin tuna from Mozambique Channel region were distinct from those captured in Seychelles-Somalia region, with depleted values in $\delta^{18}O$ relative to other nurseries. Assuming that movements of YOY away from their nursery origin are limited, the baseline sample constructed with Seychelles-Somalia and Mozambique Channel subpopulations can be used to predict the nursery origin of adolescent and adult yellowfin tuna from the western Indian Ocean. In that sense, the

results of this preliminary analysis can be regarded as promising as the methodology have allowed to discriminate among possible different spawning subpopulations (e.g. Seychelles-Somalia vs Mozambique).

Carbon and oxygen isotope values measured in otolith cores of subadult individuals indicate that the contribution of yellowfin tuna from the Mozambique Channel subpopulation to northwestern Indian Ocean is relatively low. The majority of Age 1 and Age 2 individuals captured in Somali waters were primarily derived from local production, indicating that only a small fraction of yellowfin tuna in this region originated in Mozambique Channel. This result, however, are in some extent different from the results of the IOTC Regional Tuna Tagging Programme, where a high and quick rate of mixing is evidenced across the Western Indian Ocean. Taking into account the low number of samples but a distinct signature of YOY otolith micochemistry, a more comprehensive study about otolith microchemistry is recommended to discriminate among possible different nursery areas of the western Indian Ocean, which may be expanded to the whole Indian Ocean.

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Table 1: Summary of capture location, fish number (N), mean fork length (FL) and ranges, and collection dates of young-of-the-year (YOY) yellowfin tuna (*Thunnus albacares*) collected in the western Indian Ocean by the Spanish purse seiners fleet.

Collection Area	N	Mean FL (cm)	Size-range (cm)	Collection dates
Somalia	5	40.8	34-43	July 2009
Seychelles	8	31.5	29-36	February 2009
Mozambique	10	35.4	31-37	May 2010

Table 1: Maximal classification sucess (standard deviation in parenthesis) and best combination of elements obtained with three classification methods compared: QDA: Quadratic Discriminant Analysis, RF: Random Forest and ANN: Artificial Neural Networks.

Classification method	Classification success (SD)	Combination of elements
QDA	70% (16%)	$\delta^{18}O$
RF	67% (19%)	$\delta^{13}C + \delta^{18}O$
ANN	65% (16%)	$\delta^{18}O$

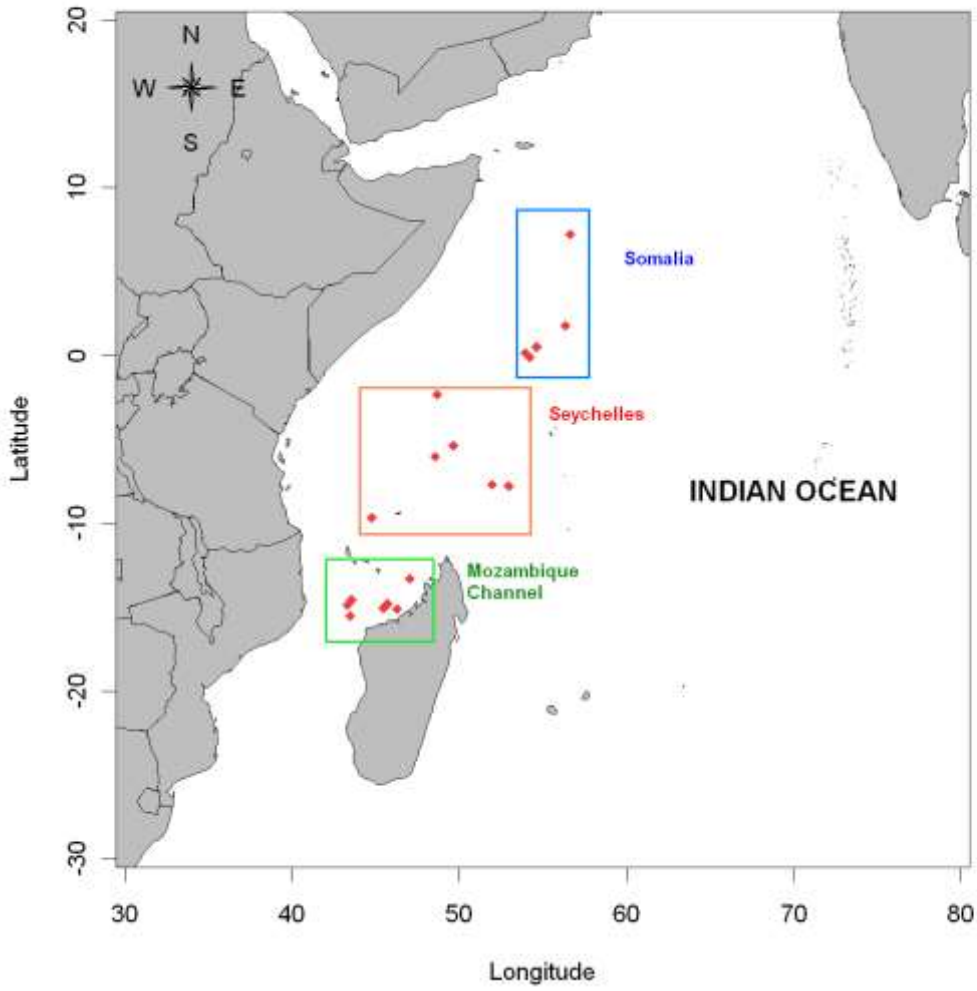

Figure 1. Location of sampling sites for yellowfin tuna (*Thunnus albacares*) in the western Indian Ocean. Three putative nurseries were identified based on the occurrence of young-of-the-year (YOY) yellowfin tuna: Somalia region (north of 3ºS), Seychelles Islands (5ºS-10ºS) and Mozambique Channel (13ºS-16ºS).

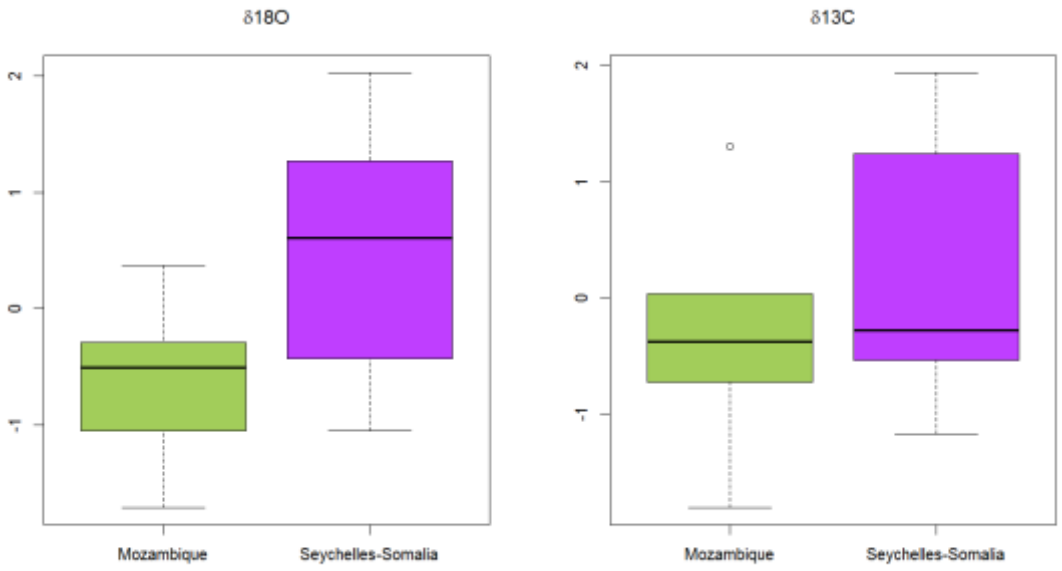

Figure 2. Boxplot of standarized $\delta^{18}O$ and $\delta^{13}C$ delta 13C and delta 18O ratios otoliths of YOY yellowfin tuna captured in Mozambique Channel and Seychelles-Somalia region between 2009 and 2010.

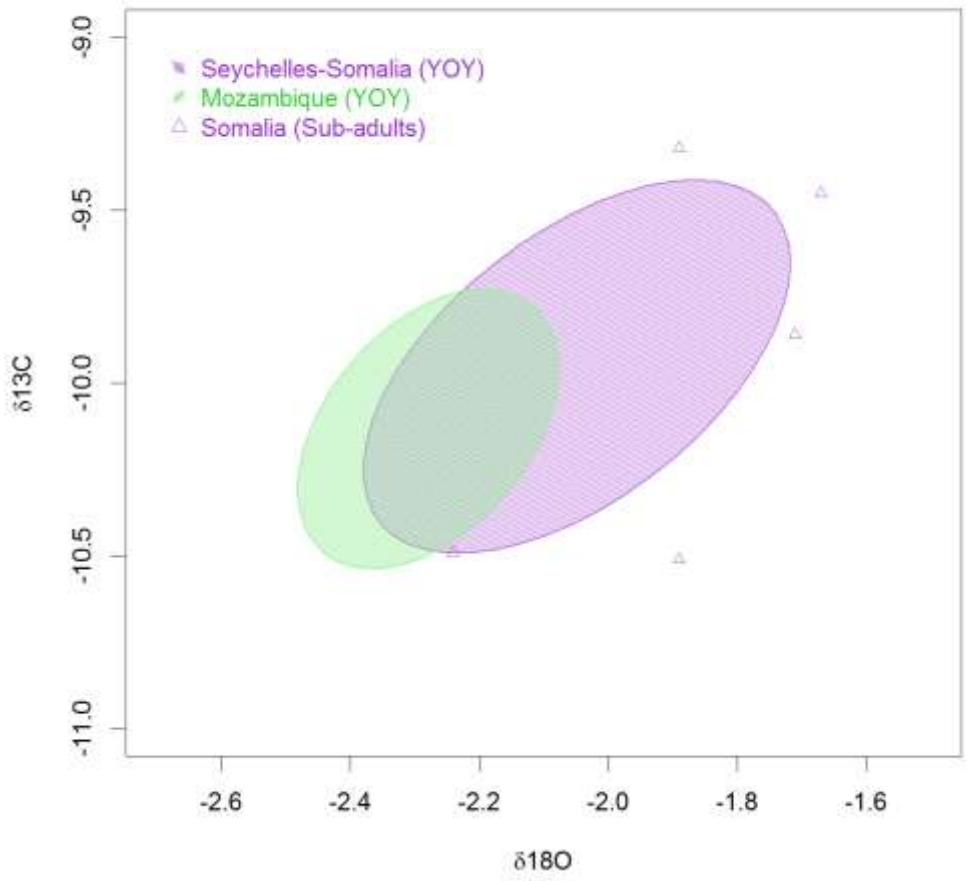

Figure 3. Otolith $\delta^{13}C$ and $\delta^{18}O$ of YFT captured in the western Indian Ocean during 2009-2010. Confidence ellipses constructed with the YOY samples from Seychelles-Somalia (purple) and Mozambique Channel (green) nurseries (1 SD or p=68%). Subadult individuals (Age 1-2) collected in Somali waters (triangles) are represented in relation to confidence ellipses.