Otolith stable isotopes suggest limited east to west connectivity of yellowfin tuna (*Thunnus albacares*) in the Indian Ocean

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Abstract

For stock assessment purposes in the Indian Ocean, a single stock of yellowfin is considered by the Indian Ocean Tuna Commission (IOTC). However, the degree of connectivity and mixing rates are still uncertain, although this information is essential for developing effective and sustainable management strategies. This study uses otolith oxygen and carbon stable isotope composition ($\delta^{18}O$ and $\delta^{13}C$) of young-of-the-year yellowfin tuna from "known" nursery areas in the equatorial Indian Ocean to establish a reference east/west baseline of isotopic signatures. This baseline was then used to determine the origin of adolescent and adult yellowfin tuna individuals captured in three fishery regions of the western Indian Ocean: R1A, R1B and R2. Results from this study suggest limited east to west connectivity of yellowfin tuna in the Indian Ocean, with west nurseries being the mayor source of contribution to the western fisheries. However, for all the three regions, we found adolescent and adult yellowfin tuna with an otolith stable isotope signal that was not characteristic of either of the two groups (east/west) present in the current baseline. This result may suggest that there is a third source of origin that is not being captured by the original baseline; being either a temporal or/and spatial component. Findings of otolith stable isotope composition of yellowfin tuna in the western Indian Ocean can provide a more comprehensive understanding of the species' spatial structure and connectivity beyond the current assessment of a single stock in the ocean basin. To that aim, advancing collaborative scientific and sampling designs in highly migratory species such as yellowfin tuna should be encouraged.

1. Introduction

Yellowfin tuna (*Thunnus albacares*) range widely across the Indian Ocean, with their habitat extending as far south as 45ºS (Sharp, 2001). However, their spawning activity is confined to regions where surface water temperatures exceed 24ºC (Schaefer, 2001). The main spawning grounds in the Indian Ocean have been described along the equatorial region (Nootmorn et al., 2005; Zhu et al., 2008; Zudaire et al., 2013). As adults, yellowfin tuna undertake extensive migrations between spawning areas in the equatorial waters and feeding grounds in southern and northern latitudes (Fonteneau and Pallares-Soubrier, 1995). Yellowfin tuna is managed as a single stock for stock assessment purposes by the Indian Ocean Tuna Commission (IOTC) (IOTC, 2021). However, the relative importance of different spawning components to the total catches, and the degree of connectivity and mixing rates of yellowfin tuna in the Indian Ocean, are still not completely understood (IOTC, 2021). This information is essential for developing effective sustainable management strategies (Kerr et al., 2016; Bosley et al., 2019) because it provides critical insights into the complexity and dynamics of fish populations. Failing to account for this complexity can lead to inaccurate population estimates by stock assessment models and, hence, to incorrect scientific management advice, which can result in either overfishing or missed opportunities for sustainable harvest (Begg et al., 1999; Ying et al., 2011).

Here we have used otolith (i.e., hard-calcareous structures found in the inner ear of fish) stable isotope ($\delta^{13}C$ and $\delta^{18}O$) composition to estimate the contribution of eastern and western spawning components to the overall catches of three fishery regions of the western (i.e., west of 75ºW) Indian Ocean used in the fleet/fishery spatial structure definition (IOTC, 2021), where the majority of yellowfin tuna catches occur.

2. Material and Methods

2.1. Sample collection

Otoliths (n=129) of yellowfin tuna were collected from three different regions (R1A, R1B and R2) of the western Indian Ocean (**Figure 1**). Fish were classified either as adolescents (43-75 cm fork length, FL) or adults (102-184 cm FL), according to the 102 cm maturity threshold in Zudaire et al. (2013) (**Table 1**). In addition, 18 otoliths of young-of-the-year (YOY, <35 cm FL) yellowfin tuna were also selected and combined with 104 YOY otoliths available from Artetxe-Arrate et al., (2021), for a total of 122 YOY otoliths available as baseline (**Table 1**). YOY yellowfin tuna were collected from three nursery areas in the western (west of 75ºE) and one nursery area in the eastern (east of 75ºE) Indian Ocean (**Figure 1**). In this study, we define nursery areas as primary regions inhabited by YOY yellowfin tuna in the Indian Ocean, which are considered important habitat during the first year of life.

2.2. Otolith stable isotope analyses

Sagittal otoliths were extracted, cleaned of adhering organic tissue, rinsed with ultrapure water, and stored dry in plastic vials. When both otoliths were available, a single sagittal otolith (right or left) was randomly selected from each pair for analysis. Detailed otolith preparation procedure can be found in (Artetxe-Arrate et al., 2021). Microsampling of otolith powder $\delta^{13}C$ and $\delta^{18}O$ stable isotope analysis was performed using a high- resolution computerised micromill (New Wave Micro - Mill System, NewWave Research). A standard template estimated to represent the material accreted during the first ~2-3 month of life was used to ensure that the same portion of the otolith was drilled in every fish. Powdered material was then analysed for $\delta^{13}C$ and $\delta^{18}O$ on an automated carbonate preparation device (KIEL-III, Thermo- Fisher Scientific) coupled to a gas-ratio mass spectrometer (Finnigan MAT 252, ThermoFisher Scientific) at the Environmental Isotope Laboratory of the University of Arizona.

2.3. Data analysis

To test the resolving power of the baseline used for this study, 8 different types of predictive model interfaces were trained: Conditional inference tree (ctree), multinomial logistic regression (MLR), K-nearest neighbors (KNN), linear support vector machine (SVM), random forest (RF), gradient boosted decision trees (xgboost), Artificial Neural Network (NNet), naïve bayes generalized linear model (BGLM) and quadratic discriminant function (QDA). Data was randomly split into 75% training and 25% testing sets, and 10-fold repeated cross validation was used for model fitting. Detailed assumptions and parameter tunning of each model can be found in Kuhn (2022).

The origin of adolescent and adult fish in the mixed sample was predicted with HISEA program and with multinomial logistic regression classification (MLR). HISEA is a commonly used statistical method for estimating the proportion of different groups in a given sample, but assumes that the probability of randomly selecting an individual from a mixed sample is known from the baseline (Millar, 1990, 1987). This may be problematic for yellowfin tuna and other highly migratory species, as the baseline data are normally sampled opportunistically, and the timing/location of the spawning is uncertain due to the lack of ocean-wide studies. Alternatively, MLR classification allows the contribution of each group to the mixed sample to be estimated, with an associated individual probability estimates (Rooker et al., 2019). For the latter, we used two probability thresholds: 0.5 and 0.8. In the first case, when an individual's predicted probability for belonging to one of the two groups in the baseline (i.e., east or west) sample is above 0.5, it will be classified to that group of origin. In the second case, when the probability of an individual belonging to a given area is below 0.8, it will be classified as unassigned (UNASS).

Finally, an additional baseline was built on simulated data. The random generation of simulated baseline data consisted of individuals with normal distribution and mean and standard deviation of $\delta^{13}C$ and $\delta^{18}O$ values equal to those of the original west and east baseline, and an alternative baseline group formed by individuals with mean and standard deviation $\delta^{13}C$ and $\delta^{18}O$ values out of the maximum ranges of the available individuals on the baseline (**Table 2**). 50 simulated individuals were generated for each of the three groups: west-like, east-like and alternative groups. This simulated dataset was then used to infer again the most likely origin of adolescent and adult fish in the mixed sample, using MLR at 0.5 probability threshold.

3. Results and discussion

Mean classification accuracy of the baseline ranged between 0.86 and 1, and kappa index between 0.68 and 1 (**Figure 2**). Therefore, the ability to discriminate between individuals from the baseline is quite high. This is mainly because individuals born in the west Indian Ocean have significantly higher $\delta^{13}C$ and $\delta^{18}O$ values in their otoliths. Otolith $\delta^{18}O$ closely reflect the ambient temperature experienced by the fish, being inversely correlated with ambient seawater temperature (Darnaude and Hunter, 2018). As the waters of the north-eastern Indian Ocean form a warm pool all year round, it is expected to find lower oxygen values in the otoliths of fish originate from this area. Nevertheless, discrimination between west and east origin fish in the baseline was not perfect. This is possibly because of the overlap in the stable isotope signal, largely due to the values of those YOY captured in Maldives region, which is a border region between west and east regions and shown intermediate values (Artetxe-Arrate et al., 2021), but that in this study has been considered as part of the baseline from the west.

Otolith core $\delta^{13}C$ and $\delta^{18}O$ values of most of the fish captured in the western region were assigned to the west origin **(Figure 3)**. In general, we found good agreement with the results obtained from both frameworks. In the R1A region, all the fish were assigned to the west origin, both with HISEA and MLR with a 0.5 probability threshold. When the threshold was increased to 0.8, 6.2% of the adolescents and 5.2% of the adults were left unassigned. In the R1B region, where only adults were available, 100% of the fish were assigned to the west both with HISEA and MLR at 0.5 probability threshold. Here, the percentage of unassigned fish increased to 31.3% when the threshold was increased to 0.8 in MLR. In the case of the adolescents from R2 region, when HISEA was used, 6.5% of the fish were assigned to the east, while no eastern origin fish were found with MLR (100% west and 80% west and 20% unassigned with 0.5 and 0.8 probability threshold, respectively). In the case of adults from R2 region, all of them were assigned to the west origin, except 3.2% unassigned with MLR at 0.8 threshold.

When looking to the assignments more in detail, many of the unassigned fish were individuals with high $\delta^{13}C$ concentrations, but with $\delta^{18}O$ values compatible with western (most of the

individuals) or eastern baseline data (**Figure 4**). However, there were some individuals, which were classified as west, but that they have an otolith stable isotope signal that was not characteristic of either of the two groups present in the current baseline (**Figure 4**). For all the three regions, there were individuals with $\delta^{13}C$ and $\delta^{18}O$ values higher to those of the baseline maximum range, particularly in R1A and R2 regions (**Figure 5**).

When individuals were re-assigned to an origin based on the simulated baseline data, most of the individuals were assigned again to the west-like origin, however, a substantial number of individuals were assigned to an alternative baseline (**Figure 6**). The proportion of individuals assigned to this alternative baseline was highest in the R1A region (45% of the fish), followed by R2 (33%) and R1B (16%). This result may suggest that there is a third source of origin that is not being captured by the original baseline (either spatial or temporal), that is contributing to the adolescents and adults fished in the western region areas. Yellowfin tuna spawning occurs mainly from November to March in equatorial waters of the western Indian Ocean, but secondary spawning peaks have also been observed from June to August (Stéquert et al., 2001; Zudaire et al., 2013a). Due to the monsoon system that governs the seasonal ocean climate in the Indian Ocean, seasonal variations in sea surface temperatures (SSTs) can be found in the tropical and subtropical regions of the western Indian Ocean; with warmer waters expected from December to March, and colder waters from June to August (Keshtgar et al., 2020). If some of the adolescents and adults sampled had been born at this second spawning peak in June-August, we would then expect higher $\delta^{18}O$ values on their otoliths, as we observed in the individuals from this alternative group. Instead, it could also be possible that yellowfin tuna assigned to this alternative group, are derived from a spawning area that was not sampled in this study. According to an $\delta^{18}O$ isoscape prediction described in Artetxe-Arrate et al. (2021), the range of $\delta^{18}O$ values observed in this alternative group, would be compatible with $\delta^{18}O$ values expected for waters off Pakistan and the Arabian Peninsula and/or waters off Reunion Islands. Recent studies using genetic methods to determine the population structure of yellowfin tuna in the Indian Ocean also suggest there are likely at least two genetic groups in the western Indian Ocean, with different contribution to samples from the north and south of the equator (Grewe et al., 2020). Using genome wide SNPs, Barth et al., (2017) also found genetic differentiation of yellowfin tuna from the Arabian Sea with respect to those from the Atlantic and Indo-Pacific. Likewise, genetic studies based on mitochondrial DNA, suggested the existence of discrete yellowfin tuna populations in the north-central Indian Ocean (Dammannagoda et al., 2008; Kunal et al., 2013). All these genetics studies support a more complex population structure than the single stock hypothesis currently considered for the management of the species, as did our findings suggesting a possible alternative spawning area for those individuals not assigned to the original west/east baseline.

Overall, results from this study suggest limited east to west connectivity of yellowfin tuna in the Indian Ocean. However, due to the lack of adolescent and adult samples from the eastern region, it has not been possible to investigate the contribution of western nursery areas to eastern fisheries, and thus the degree of connectivity at oceanic scale. Efforts should be directed towards obtaining a good representation of otolith samples from both sides of the Indian Ocean. Moreover, the combination of both otolith chemistry data and genetic markers should be explored, as each technique covers different aspects of the biology of the species and provide different spatio-temporal resolution on species degree of connectivity. Therefore, this combined approach can provide a more holistic understanding of ecological and evolutionary processes and may identify stock units with higher degree of confidence, and/or unravel otherwise hidden patterns of connectivity. Finally, this information can have important implications for yellowfin tuna management purposes in the Indian Ocean; help at defining conservation efforts and increase our understanding on the impact of fishing pressure and environmental changes on the species.

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Table 1. Number, age class, size and sampling period of yellowfin tuna (*Thunnus albacares*) at each sampling region or nursery. Size is fork length (FL) in cm. YOY: Young-of-the.year.

Region/Baseline	n	Age class	FL-range	Sampling year
West baseline	79	YOYs	26-35	2018-2019
East baseline	43	YOYs	19.5-34	2018
R1A	32	Adolescents	43-75	2018 and 2021
R1A	19	Adults	102-184	2021
R1B	32	Adults	103-144	2021-2022
R2	15	Adolescents	47-50.5	2017
R2	31	Adults	124-169	2018-2019

Table 2. Mean and standard deviation (sd) $\delta^{13}C$ and $\delta^{18}O$ values used to randomly generate simulated baseline data. For each group 50 individuals were created based on these values for the normal distribution.

Group	Mean $\delta^{13}C$	sd $\delta^{13}C$	Mean $\delta^{18}O$	sd $\delta^{18}O$
West-like	-10.50	0.40	-1.98	0.28
East-like	-11.10	0.40	-2.56	0.30
Alternative	-9.50	0.40	-1.00	0.30

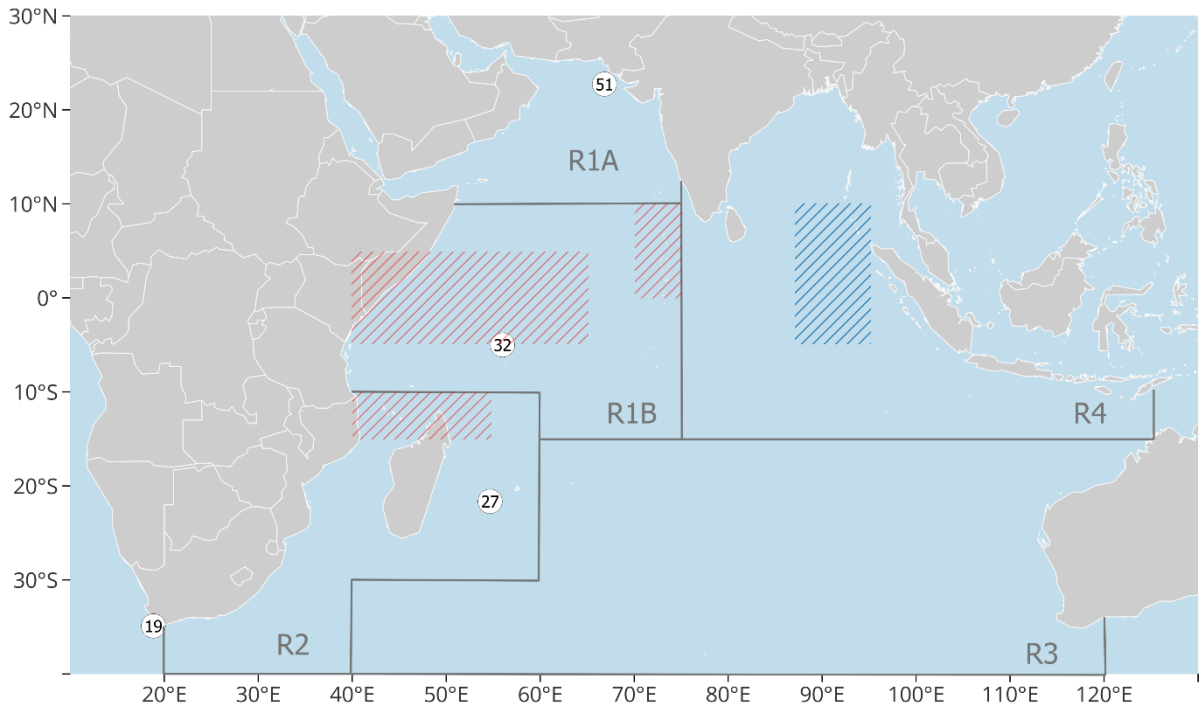

Figure 1. Map showing approximate sampling locations for adolescent and adult yellowfin tuna (*Thunnus albacares*) otoliths collected for origin assignment (white dots, numbers of individuals are shown). Lined squares represent nursery areas were YOY have been caught and used as baseline individuals for the west (red) and east (blue) Indian Ocean. Regions are defined after (IOTC, 2021); R1A (Arabian Sea), R1B (Off Somalia), R2 (Mozambique Channel, including southern), R3 (South Indian Ocean including southern), R4 (East Indian Ocean, including Bay of Bengal).

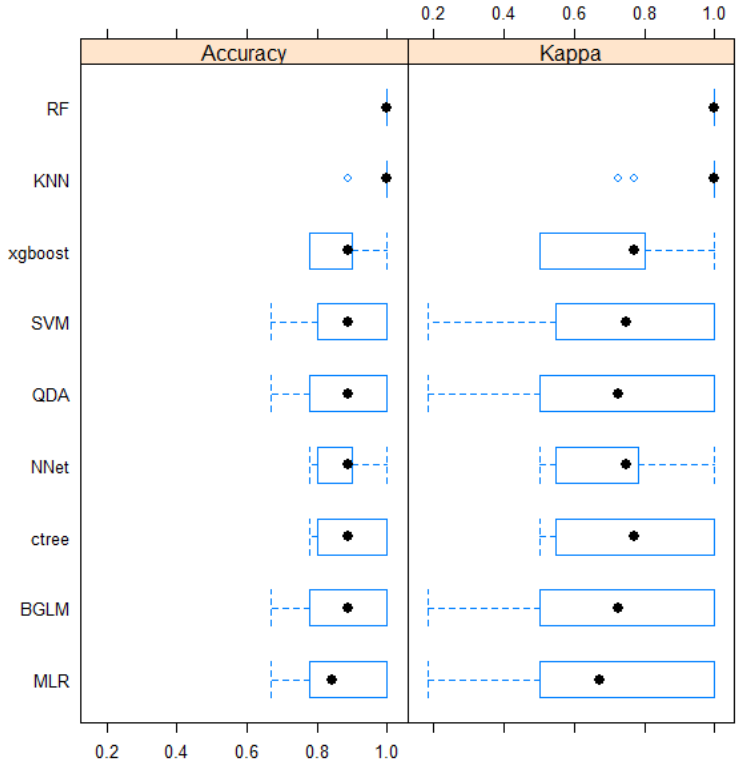

Figure 2. Baseline data classification accuracy (left panel) and kappa index (right panel) in assigning YOY yellowfin tuna (*Thunnus albacares*) to their nursery region of origin (East or West). Training models are: Conditional inference tree (ctree), multinomial logistic regression (MLR), K-nearest neighbors (KNN), linear support vector machine (SVM), random forest (RF), gradient boosted decision trees (xgboost), Artificial Neural Network (NNet), naïve bayes generalized linear model (BGLM) and quadratic discriminant function (QDA).

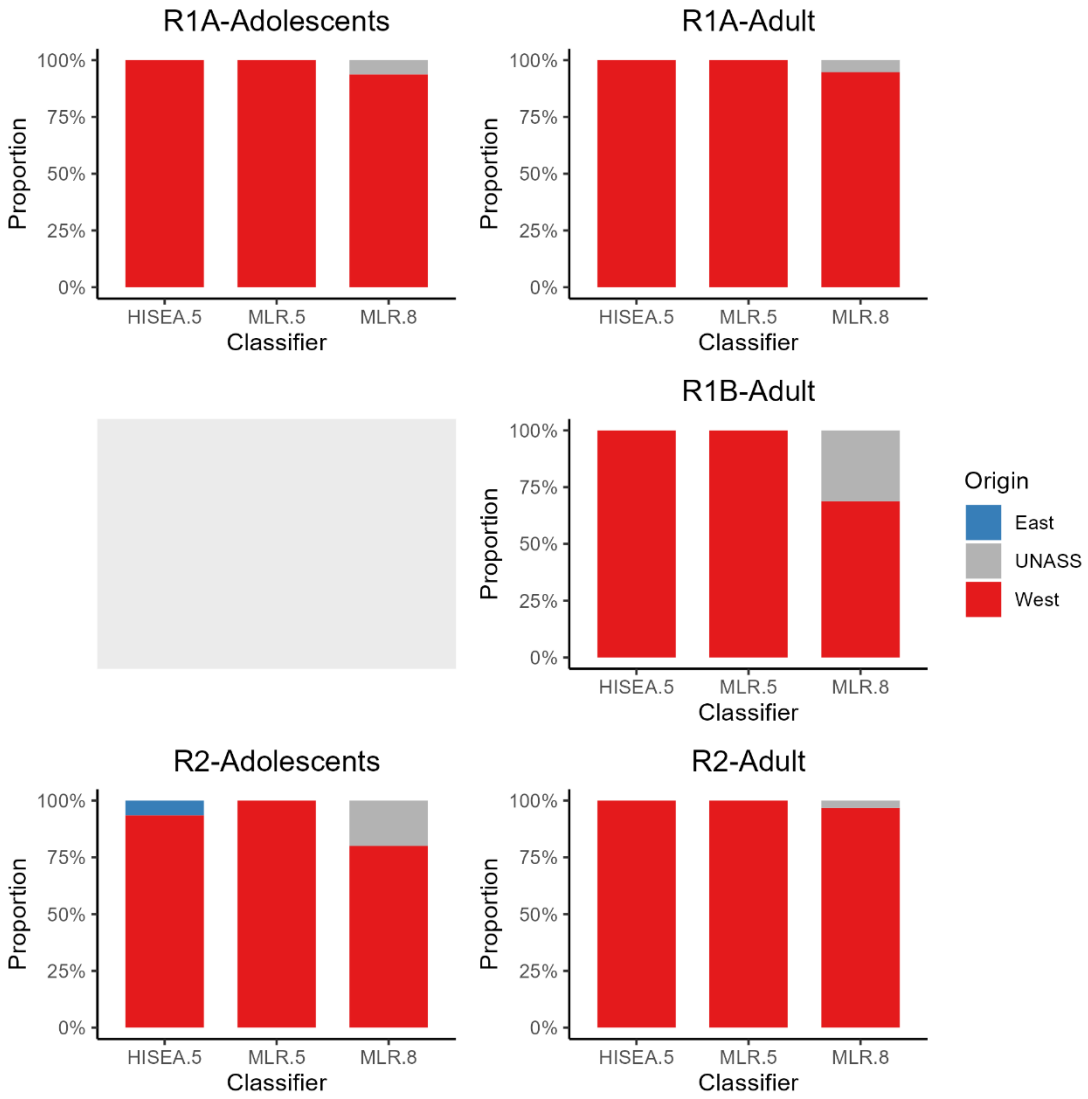

Figure 3. Estimated proportions of western (red), eastern (blue) and unassigned (grey) adolescent and adult yellowfin tuna (*Thunnus albacares*) captured in the western Indian Ocean. Results are based on mixed population analysis using HISEA with a decision threshold of 0.5 (HISEA.5) and multinomial logistic regression with a decision threshold of 0.5 (MLR.5) and 0.8 (MLR.8)

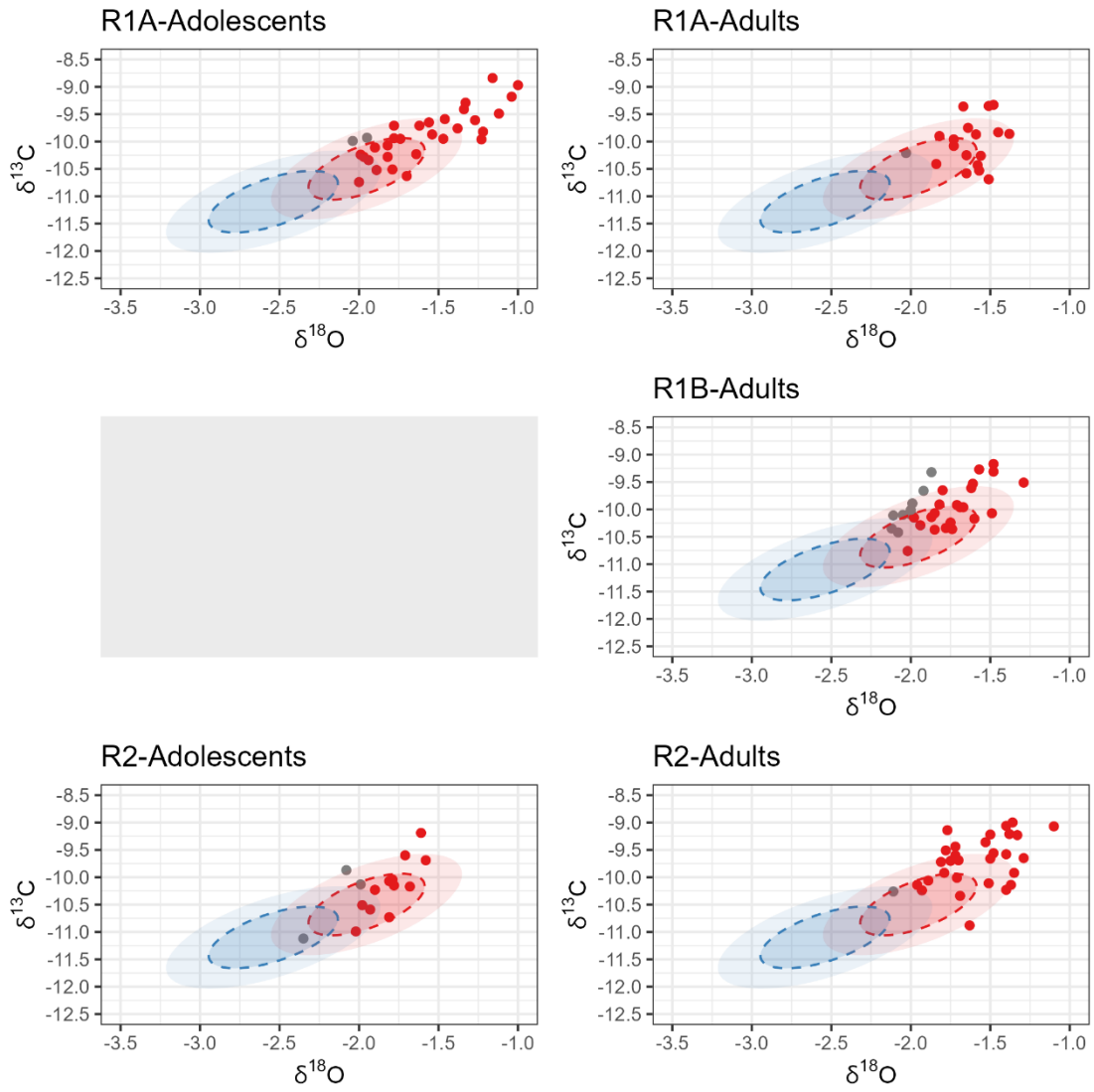

Figure 4. Otolith $\delta^{13}C$ and $\delta^{18}O$ values of adolescent and adult yellowfin tuna (*Thunnus albacares*) collected in R1A, R1B and R2 fishing regions of the Indian Ocean. Colour of the dots represent to which group they have been assigned to based on the MLR 0.8 framework: west (red), east (blue) or unassigned (grey). Confidence ellipses shown are based on otolith $\delta^{18}O$ and $\delta^{13}C$ values of YOY from the west (red) and east (blue) baseline and represent p=0.68 (1 SD) and p=0.95 confidence intervals.

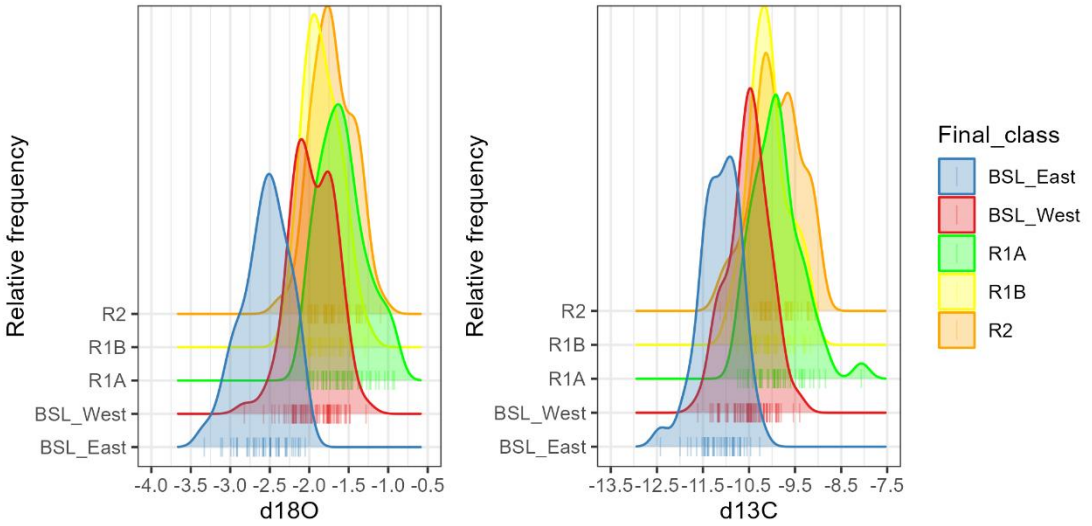

Figure 5. Relative density of $\delta^{18}O$ (left panel) and $\delta^{13}C$ (right panel) values of YOY yellowfin tuna (*Thunnus albacares*) used as east (blue) and west (red) baseline, as well as of adolescent and adult individuals collected in R1A (green), R1B (yellow) and R2 (orange) fishing regions of the Indian Ocean.

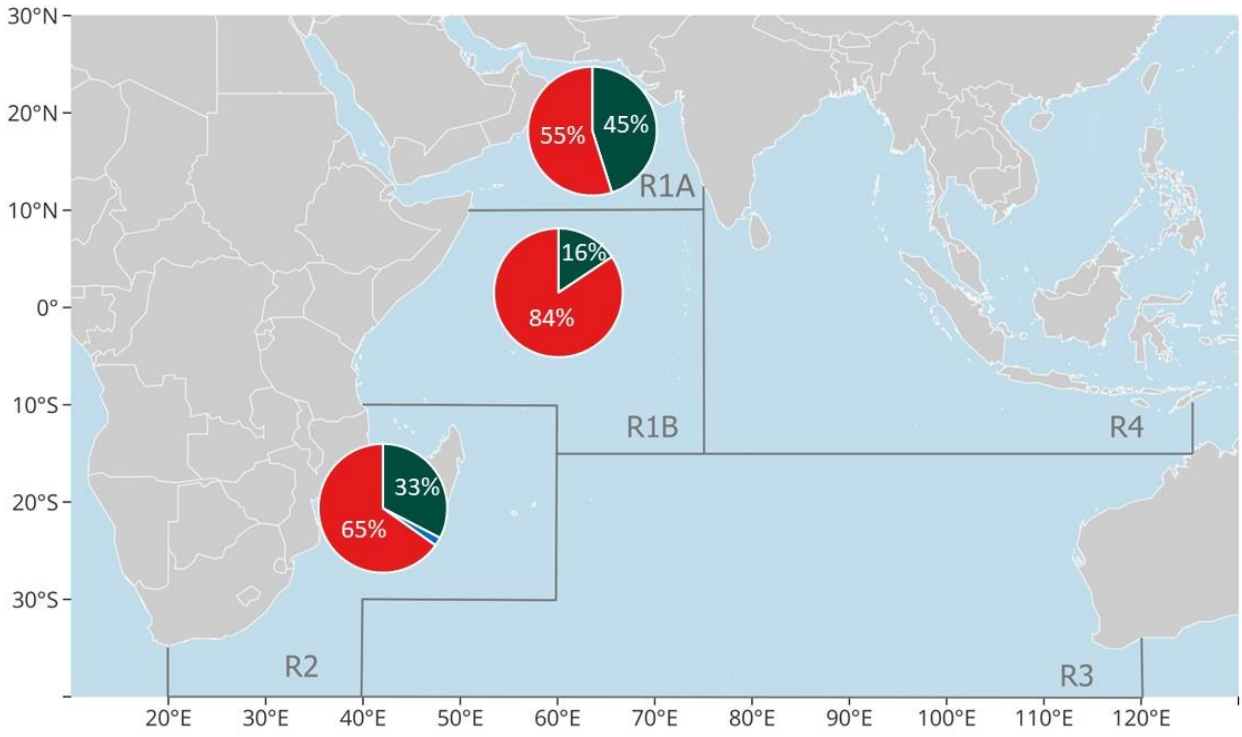

Figure 6. Origin assignment of adolescent and adult yellowfin tuna (*Thunnus albacares*) captured in R1A, R1B and R2 fishing regions of the Indian Ocean, after using a simulated baseline (n=150). Proportion of samples assigned to West-like baseline (red), East-like baseline (blue) and Alternative baseline (dark green) are shown. Values indicate the % of samples assigned to each origin.