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A close-kin mark-recapture pilot study for Indian Ocean yellowfin tuna

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Contents

1 Summary

A close-kin mark-recapture (CKMR) design study completed in 2022 estimated that the collection of approximately 30,000 samples per year from Indian Ocean yellowfin tuna, over a five-year period, would provide an estimate of absolute abundance with an acceptable level of precision. The Working Party on Methods and Working Party on Tropical Tunas noted the logistical challenges in collecting this many samples and suggested a staged approach to the implementation of CKMR for yellowfin tuna. This paper outlines a proposal for the implementation of a CKMR pilot project for Indian Ocean yellowfin tuna to evaluate the logistics and feasibility of sampling, including as assessment of the quality of the DNA collected from key locations. The Scientific Committee is invited to provide feedback on this proposal.

2 Introduction

Uncertainty in tuna stock assessments across Regional Fisheries Management Organisations (RFMOs) has motivated the exploration of alternative approaches for estimating abundance of tuna. This is particularly the case for the Indian Ocean yellowfin tuna stock, which has been estimated to be overfished and subject to overfishing since 2015, and was recently subject to an external review due in part to conflicts and inaccuracies in the data inputs to the stock assessment (Maunder et al. 2023).

Close-kin mark-recapture (CKMR) is a fisheries-independent method that can provide an estimate of absolute abundance and other key population metrics such as total mortality (Bravington et al. 2016a). Importantly, it is independent of the main data inputs and biases associated with the current stock assessment. CKMR has a proven track record in the assessment and management of southern bluefin tuna (Bravington et al. 2016b), where the data are integrated into the stock assessment models and are used in the management procedure (e.g., Hillary et al. 2019, 2020). CKMR is being developed for a range of other species, and across the tuna RFMOs, including for Atlantic bluefin tuna (Grewe et al. 2018) and South Pacific albacore (SPC-OFP and CSIRO 2023).

CKMR was first identified as a priority research activity for yellowfin tuna in the Working Party on Tropical Tuna (WPTT) Program of Work in 2017 (IOTC 2017). In 2022, a design study for Indian Ocean yellowfin tuna evaluated a range of sampling scenarios that would provide estimates of spawning stock biomass, depletion, adult mortality and mean recruitment using CKMR (Hillary et al. 2022). The authors concluded that annual sample sizes of approximately 30,000 collected from the catch of yellowfin tuna over a five-year period would provide reasonable precision in estimates of these population metrics. Furthermore, they estimated that greater precision in population parameter estimates could be achieved by sampling proportionally more juveniles than adults, specifically 70% juveniles and 30% adults.

In 2022 and 2023, the Working Party on Methods (WPM) and WPTT supported further advancing the implementation of CKMR for Indian Ocean yellowfin tuna (IOTC 2022a, b). The WPM and WPTT noted the logistical challenges in collecting the recommended number of samples, and suggested that a staged approach be used, whereby an initial year of sampling is conducted to determine whether samples in sufficient numbers and quality can be collected while maintaining DNA contamination at acceptably low levels. The WPM and WPTT also noted the possible need for adaptive sampling, whereby the annual sample size may need to increase in subsequent years if the early sampling contains fewer parent-offspring pairs (POPs) and half-sibling pairs (HSPs) than expected based on the results of the design work.

This paper outlines a proposal for the implementation of a CKMR pilot project for Indian Ocean yellowfin tuna to evaluate the logistics and feasibility of sampling, and levels of cross-contamination of DNA. CKMR requires an estimate of age for the sampled individuals to determine birth year and age-specific parameters for the calculation of kinship probabilities. Therefore, this proposal also contains components to refine estimates of age-specific reproductive biology and to develop epigenetic ageing methods specific to Indian Ocean yellowfin tuna. Otoliths currently provide the most reliable estimates of age for tunas, but it is unlikely that 30,000 otoliths could be collected, processed and analysed each year for yellowfin tuna. Epigenetic ageing methods using DNA methylation have demonstrated encouraging results across a range of species (Mayne et al. 2020, 2021), including yellowfin tuna in the Pacific Ocean up to 10 years of age (Mayne et al. 2023). The cost of epigenetics is comparable to using otoliths to estimate age, but tissue samples for epigenetics can be collected far more easily than otoliths. Epigenetic ageing would present a more viable option for CKMR sampling of yellowfin tuna as the same tissue sample could be used for both genotyping (to identify kin pairs) and epigenetic ageing.

3 Proposed pilot study

3.1 Objectives

The proposed CKMR pilot study for Indian Ocean yellowfin tuna is a 3-year project with the main objectives to:

1. Establish a network of international collaborators to coordinate the implementation of a sampling program for yellowfin tuna.
2. Develop standard operating procedures for collecting, storing and transporting yellowfin tuna tissue samples.
3. Collect up to the annual target of 30,000 muscle tissue samples from yellowfin tuna from across the Indian Ocean, aiming for approximately 70% juvenile and 30% adult fish.
4. Examine the level of DNA contamination among samples collected and adjust sample collection methods if necessary.
5. Calibrate an epigenetic clock to estimate the age of Indian Ocean yellowfin tuna from tissue samples (Mayne et al. 2023).
6. Refine estimates of age-specific maturity and fecundity for Indian Ocean yellowfin tuna.
7. Evaluate the feasibility of implementing the full 5-year sampling design recommended by Hillary et al. (2022).

3.2 Schedule

Year 1

Several workshops with key international collaborators would be scheduled in the first year of the project to establish the project team and institutional arrangements, and to develop standard operating procedures for the training and sampling. Training of personnel (e.g. port samplers, observers) to conduct the sampling will also commence in the first year.

Age-specific estimates of maturity and fecundity and an epigenetic clock for Indian Ocean yellowfin tuna will be developed in the first year of the project from available gonad and tissue samples that have been collected during previous projects such as the GERUNDIO project (Farley et al. 2023). Additional samples collected in the second year of the project may be required to supplement the gonad samples and extend the age range of samples for the epigenetic clock if the initial samples are insufficient or do not cover the full age range expected for yellowfin tuna.

Year 2

The sampling program will commence in the second year, with the aim to collect up to the target of 30,000 samples consisting of approximately 70% juveniles (<50 cm) and 30% adults (>75 cm)

The sampling program will be reviewed after the completion of 12 months and reported back to the WPM, WPTT and Scientific Committee (SC) to evaluate the feasibility of collecting the target number of samples. If collecting the target number of 30,000 samples looks feasible, sampling will continue in the third year of the project with the aim of supporting the full implementation of CKMR for yellowfin tuna.

Year 3

The level of cross-contamination of DNA in the samples will be tested for a subsample of fish collected from each major source of sampling. If levels of contamination are found to be high, adjustments to the sampling methods will be made for future sampling.

Results from the CKMR pilot study for Indian Ocean yellowfin tuna will be reported back to the WPM, WPTT and SC at the conclusion of the project, where a decision to support the continuation of CKMR for this population will be made.

If a full CKMR study was to proceed following the pilot study, the samples collected therein could be used in the full study. Adequate storage for all samples collected would be included as part of the project.

3.3 Budget

The cost of implementing this CKMR pilot study for Indian Ocean yellowfin tuna is substantially less than the full implementation of CKMR, as only 1-2 years of samples will be collected, as opposed to 5 years, and most of these samples will not be genotyped (at the pilot program stage). However, there are still significant costs in coordinating the collection of a large number of samples across the Indian Ocean. Therefore, it will be necessary to source funds external to the IOTC science budget. An approximate budget for the implementation of this CKMR pilot study is likely to be around USD$2M over a period of three years. A more detailed budget will be developed for a formal funding proposal if supported by the SC.

3.4 Sampling considerations

Obtaining an adequate spread of samples across time and space will be important to account for any population structuring, movement, and spatial variation relevant to stock parameters. A mix of size classes of yellowfin tuna will also be required to ensure that both potential offspring and parents are adequately sampled, and the juveniles sampled will need to be young enough to infer their region of origin.

Collecting tissue samples from fish that are already measured as part of an ongoing data collection program is likely to be easier than establishing supplementary tissue sampling programs. Therefore, the fleets and fisheries that will most likely be of utility in undertaking large-scale sampling programs for the purposes of CKMR are those currently providing length data through observer or port sampling programs.

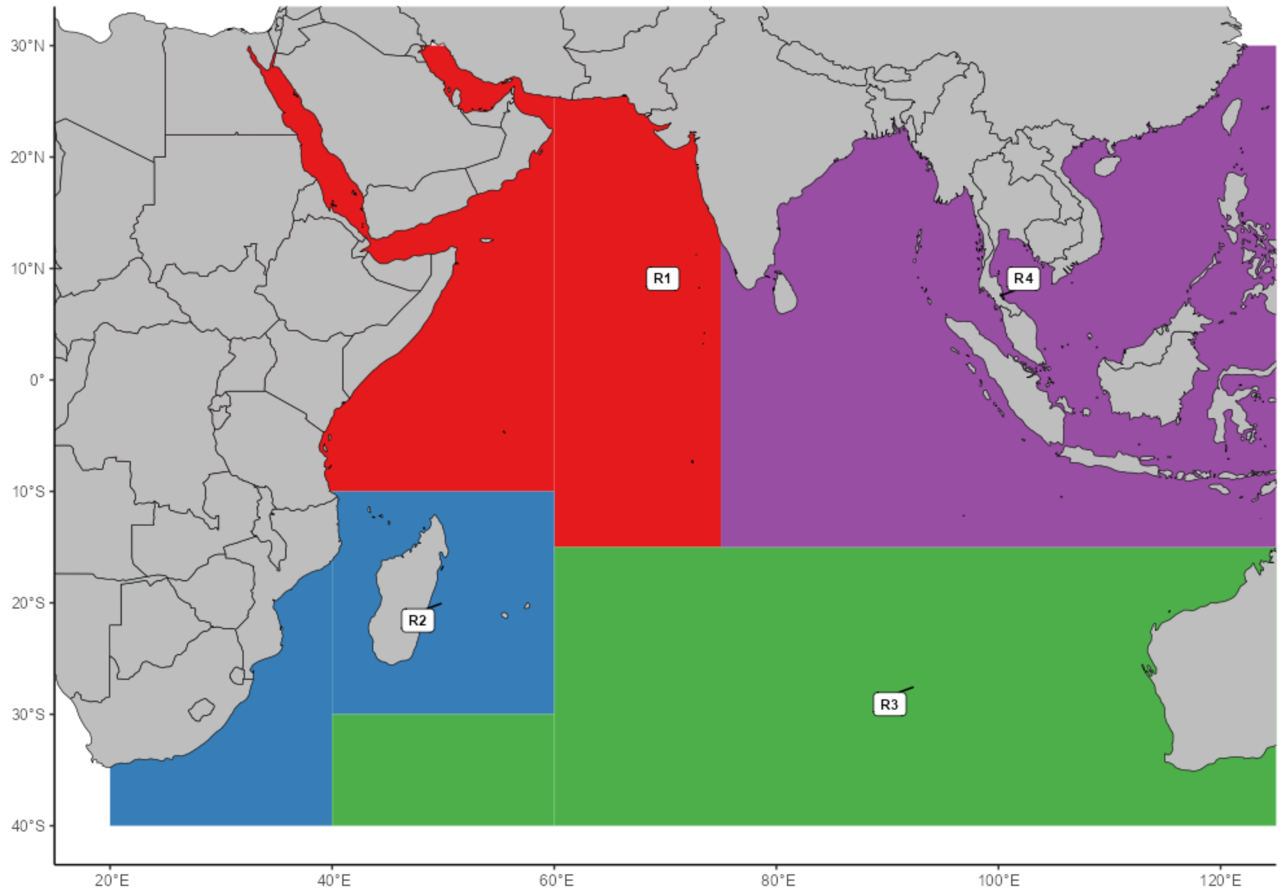

Figure 1. Four region spatial stratification of the Indian Ocean for the basic yellowfin tuna assessment model (Fu et al. 2021).

The potential to collect sufficient samples of yellowfin tuna for CKMR can be evaluated by examining the length data reported to the IOTC. Using the four stock assessment areas to spatially spread the sampling (Figure 1), and two size classes (<50 cm and >75 cm) to represent the juvenile and adult samples, two strong modes in the length frequency data are apparent (Figure 2) that reflect the difference in targeting and selectivity of gear types, and the distribution of juvenile yellowfin tuna. Most length data have been reported from Region 1 and Region 4 and these are also the regions where the largest numbers of small yellowfin tuna (<50cm) are reported. This is to be expected as yellowfin tuna spawn in tropical, equatorial waters and juveniles are less common in more temperate waters. Large yellowfin tuna (>75cm) are routinely reported in all regions except for Region 3 where sampling is relatively sparse due to substantially reduced fishing effort in this region.

Results from the CKMR design study suggest that approximately 30,000 samples of yellowfin tuna per year over five years would be required to achieve adequate acceptable levels of precision for key population metrics, with optimal precision achieved by sampling 70% juveniles and 30% adults. Given this information, and if uniform sampling was assumed across the four regions and two size classes, this would require around 2,250 large yellowfin tuna (>75cm) sampled each year from each region, and 10,500 small yellowfin tuna (<50cm) from Region 1 and Region 4, as very few juveniles are likely to be sampled from Region 2 and Region 3.

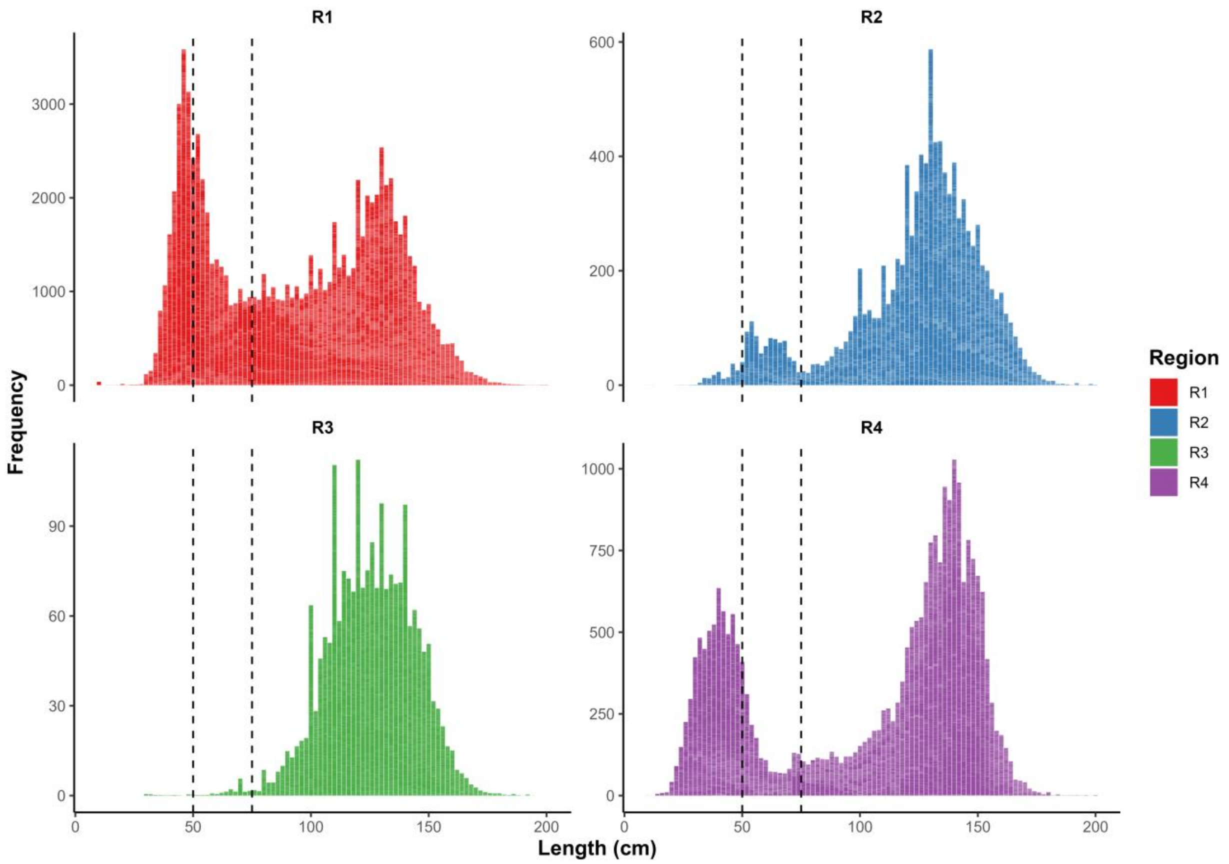

Figure 2. Average annual length frequency distribution of sampled Indian Ocean yellowfin tuna by region (panels) from 2018-2022 with two vertical dashed lines denoting potential offspring (<50cm) and parents (>75cm). Note independent y-axes.

This level of sampling intensity is already achieved across most region and size class combinations in the length data collection programs currently implemented (Figures 3 & 4). For small yellowfin tuna (<50cm), more than 10,000 individuals have been measured annually on average in Region 1 and Region 4 from 2018-2022 from the top 3 fleets reporting length data (Figure 3). For large yellowfin tuna (>75cm), more than 2,000 individuals have been measured annually on average from 2018-2022 across all regions by the top 3 fleets reporting length data, although the sampling intensity in Region 3 is much lower than other regions (Figure 4).

An informed and substantial sampling program will be required to collect this number of samples across regions and size classes throughout the Indian Ocean. The key fleets collecting length data across all regions include Maldives, EU Spain, EU France, Seychelles, Indonesia, Sri Lanka, Iran, Japan and Taiwan,China. Therefore, a successful sampling program will require collaboration and cooperation from numerous member nations, institutes, organisations and, most importantly fishers, observers, and port samplers. The proposed workshops for this CKMR pilot project will bring together expert knowledge of the fisheries operating across the Indian Ocean and of the potential sampling opportunities (e.g., port sampling, observers, etc.).

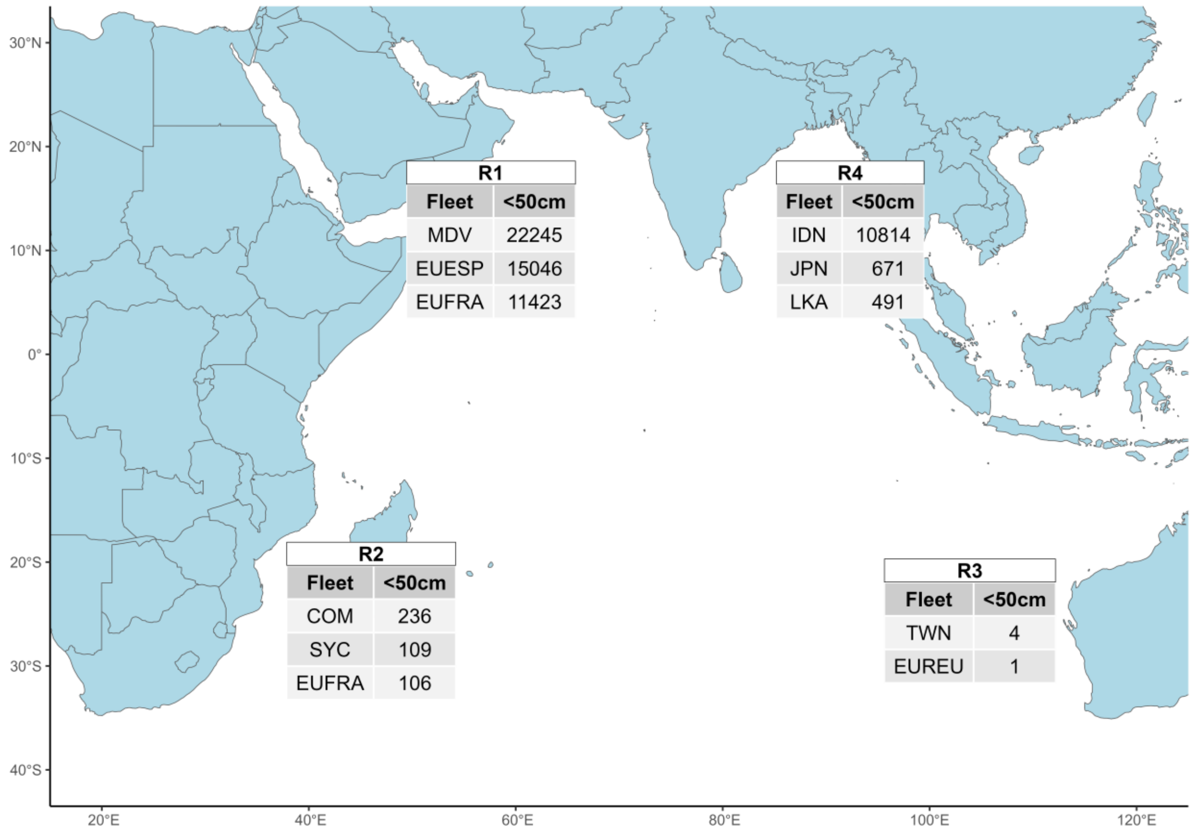

Figure 3. Average annual number of small yellowfin tuna (<50 cm FL) length measurements reported in the 5 years from 2018-2022 in each of the four stock assessment regions in the Indian Ocean by the top three fleets that provide length data.

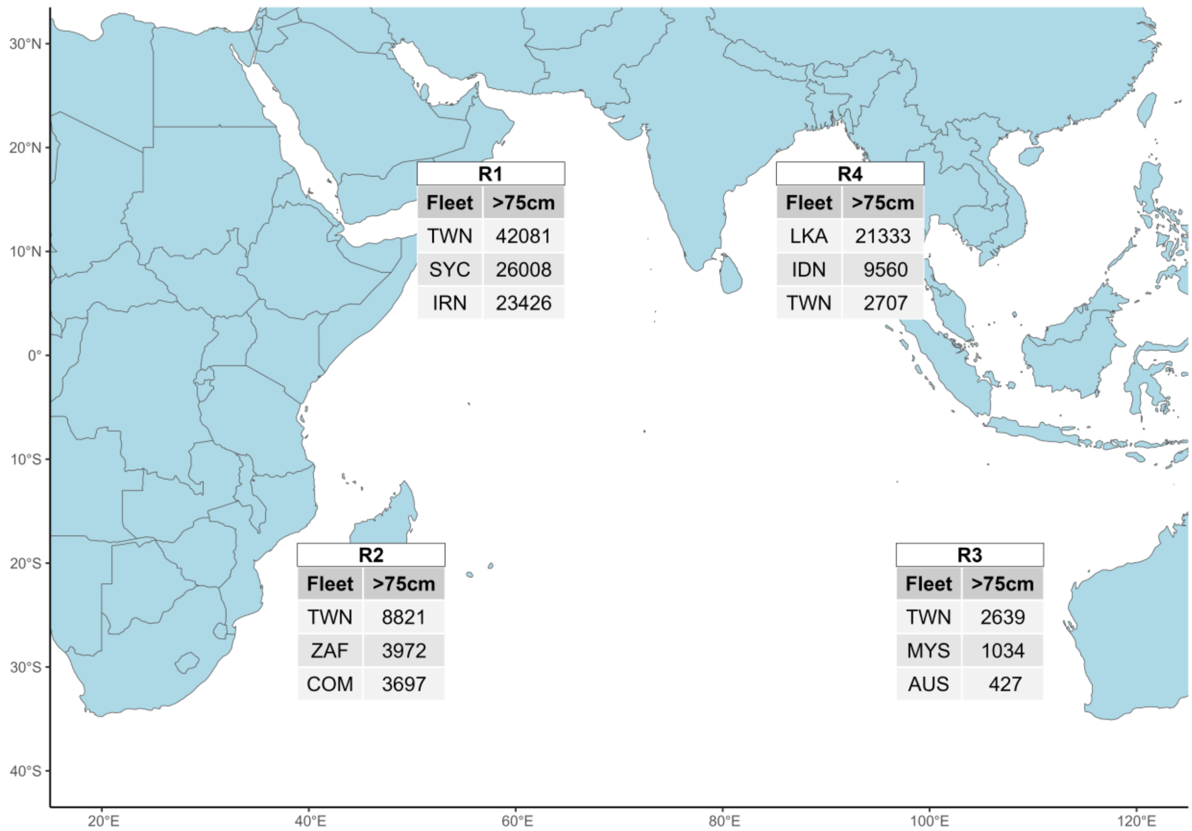

Figure 4. Average annual number of large yellowfin tuna (>75 cm FL) length measurements reported in the 5 years from 2018-2022 in each of the four stock assessment regions in the Indian Ocean by the top three fleets that provide length data.

3.5 Sample collection & processing

The development of standard operating procedures for sampling will be an important product developed during the proposed workshops in the first year of the project. These standard operating procedures will outline the protocols for samplers to use when identifying species (as juvenile yellowfin tuna are difficult to differentiate from juvenile bigeye tuna), selecting which fish to sample, collecting tissue samples, recording ancillary data (including accurate length measurement), storing tissue samples and shipping samples to the laboratory for DNA extraction and potential sequencing. The standard operating procedures are particularly important to ensure that cross-contamination of DNA is minimised, as contamination increases the rate of false positives in the kin-finding process of CKMR.

Tissue samples collected during the first year of sampling will be sent to a laboratory at CSIRO in Hobart, Australia for storage. DNA will be extracted for a subsample of 50 fish collected from each major location of sampling and used to test for cross-contamination. The level of cross-contamination of DNA in the samples will be examined to identify sampling methods, locations or samplers for which contamination might be unacceptably high. If levels of contamination are found to be high, adjustments to sampling methods will be made for future sampling.

Gonad samples and data collected during previous biological projects for Indian Ocean yellowfin tuna, such as the GERUNDIO project (Zudaire et al. 2021), will be used to refine the age-specific maturity schedule and fecundity-at-age estimates. If existing sample collections are insufficient to provide robust estimates of these parameters, then additional gonad samples will be collected during the first year of sampling of this pilot project.

An epigenetic clock has been developed for yellowfin tuna in the Pacific Ocean, but it is unclear whether it is appropriate to apply this to Indian Ocean yellowfin tuna. Therefore, in the first year of the project, an epigenetic clock will be developed using direct age data from otoliths and tissue samples collected from Indian Ocean yellowfin tuna during previous projects (e.g. Farley et al. 2023). Targeted sampling of otoliths and tissue from large (and potential old) fish during the first year of sampling will also be undertaken if needed to extend the age range for the epigenetic clock. The epigenetic clock will then be available to estimate the age of all samples collected in the full implementation of CKMR for Indian Ocean yellowfin tuna.

4 Discussion

This paper details a possible pilot study to support the implementation of close-kin methods for unbiased estimation of population abundance and other management metrics of yellowfin tuna in the Indian Ocean. It responds to concerns by the WPM and WPTT that the number of samples required annually might be logistically challenging.

The approach outlined here would lead to an improved understanding of logistical hurdles in the large-scale collection of tissue samples for yellowfin tuna in the Indian Ocean. It would allow the WPM, WPTT and SC to make an informed decision when evaluating whether to pursue a full CKMR study. If a full CKMR study was to proceed following the pilot study, the samples collected therein could serve as the first 1-2 years of the required samples. Conversely, if the SC decided not to support a full CKMR study, this project will have provided a calibrated epigenetic clock to facilitate the ageing of yellowfin tuna individuals, refined estimates of maturity and fecundity, as well as enhanced regional capacity for large-scale sampling of this stock through training, network development and documentation of sampling opportunities. Given ongoing issues in the quality of data inputs to the current stock assessment, such opportunities for large-scale sampling have applications beyond CKMR.

As such, the authors seek support and feedback from the SC on the proposed pilot study (noting funding to be sourced externally to IOTC), as well as expressions of interest for collaboration in the development of international networks to conduct large-scale sampling for yellowfin tuna.

5 References

Bravington, M.V., Skaug, H.J., Anderson, E.C. 2016a. Close-Kin Mark-Recapture. *Statistical Science* 2016, Vol. 31(2), 259–274.

Bravington, M.V., Grewe, P.M., Davies, C.R. 2016b. Absolute abundance of southern bluefin tuna estimated by close-kin mark-recapture. Nature Communications 7:13162.

Farley, J., Krusic-Golub, K., Eveson, P., Luque, P.L., Fraile, I., Artetxe-Arrate, I, Zudaire, I., Romanov, E., Shahid, U., Razzaque, S.A., Parker, D., Clear, N., Murua, H., Marsac, F., Merino, G. 2023. Updating the estimation of age and growth of yellowfin tuna (*Thunnus albacares*) in the Indian Ocean using otoliths. Working paper IOTC–2023–WPTT25–20 presented to the 25th Working Party on Tropical Tuna, San Sebastian, Spain 30 October – 4 November 2023.

Fu, D., Ijurco, A.U., Cardinale, M., Methot, R., Hoyle, S. & Merino, M. 2021. Preliminary Indian Ocean yellowfin tuna stock assessment 1950-2020 (Stock Synthesis). Working paper IOTC–2021–WPTT23–12 presented to the 23rd Working Party on Tropical Tuna.

Grewe, P., McDowell, J., Walter, J., Lauretta, M., Gosslien, T., Bravington, M.V., Porch, C., Davies, C.R. 2018. Genomic tools demonstrate excellent potential for estimating a census estimate for Atlantic Bluefin Tuna spawning in the Gulf of Mexico. ICES ASC.

Hillary, R., Preece, A., & Davies, C. 2019. Performance of a revised candidate MP using all 3 input data sources. Paper CCSBT-ESC/1909/16 prepared for the Extended Scientific Committee for the Twenty Fourth Meeting of the Scientific Committee. Commission for the Conservation of Southern Bluefin Tuna.

Hillary, R., Preece, A., & Davies, C. 2020. Summary of updated CKMR data and model performance in the Cape Town Procedure. Paper CCSBT-ESC/2008/BGD 07 prepared for the Extended Scientific Committee for the Twenty Fifth Meeting of the Scientific Committee. Commission for the Conservation of Southern Bluefin Tuna.

Hillary, R. M., Tremblay-Boyer, L., Williams, A.J., Hill, N.J., Preece, A.L. 2022. Indian Ocean yellowfin tuna close-kin mark-recapture design study. Working Paper prepared for the 13th IOTC Working Party on Methods, 19-21 October 2021.

IOTC 2017. Report of the 20th Session of the IOTC Scientific Committee. 30 November – 4 December 2017. IOTC–2017–SC20–R[E], Seychelles.

IOTC 2022a. Report of the 13th Session of the IOTC Working Party on Methods. Held by videoconference, 19 – 21 October 2022. IOTC–2022–WPM13–R.

IOTC 2022b. Report of the 24th Session of the IOTC Working Party on Tropical Tunas. Held by videoconference, 24 – 29 October 2022. IOTC–2022–WPTT24–R.

Maunder, M., Langley, A., Howell, D., Minte-Vera, C. 2023. Independent review of recent IOTC yellowfin tuna assessment. Working paper IOTC-2023-WPTT25-13 presented to the 25th Working Party on Tropical Tuna, San Sebastian, Spain 30 October – 4 November 2023.

Mayne, B., Korbie, D., Kenchington, L., Ezzy, B., Berry, O. & Jarman, S. 2020. A DNA methylation age predictor for zebrafish. *Aging* (Albany NY), 12(24), 24817.

Mayne, B., Espinoza, T., Roberts, D., Butler, G.L., Brooks, S., Korbie, D. & Jarman, S. 2021. Nonlethal age estimation of three threatened fish species using DNA methylation: Australian lungfish, Murray cod and Mary River cod. *Molecular Ecology Resources*, 21(7), 2324-2332.

Mayne, B., Lloyd-Jones, L., Anderson, C., Bravington, M., Aulich, J., Potter P., Farley, J., Davies, C. 2023. Update on epigenetic ageing. Information paper prepared for the 14th IOTC Working Party on Methods, 26-28 October 2023.

SPC-OFP and CSIRO. 2023. Progress towards a close-kin-mark-recapture application to South Pacific albacore (project 100c). Working Paper prepared for the 19th Session of the WCPFC Scientific Committee, 16-24 August 2023, Koror, Palau. WCPFC-SC19-2023/SA-WP-13.

Zudaire, I., Artetxe-Arrate, I., Farley, J., Murua, H., Kukul, D., Vidot, A., Razzaque, S.A., Ahusan, M., Romanov, E., Eveson, P., Clear, N., Luque, P.L., Fraile, I., Bodin, N., Chassot, E., Govinden, R., Ebrahim, A., Shahid, U., Fily, T., Marsac, F., Merino, G. 2021. Preliminary estimates of sex ratio, spawning season, batch fecundity, length at maturity for Indian Ocean yellowfin tuna. Information paper IOTC-2021-SC24-INF01 submitted to the 24th Session of the IOTC Scientific Committee.

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