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Summary of population structure of IOTC species from PSTBS-IO project and recommended priorities for future work.

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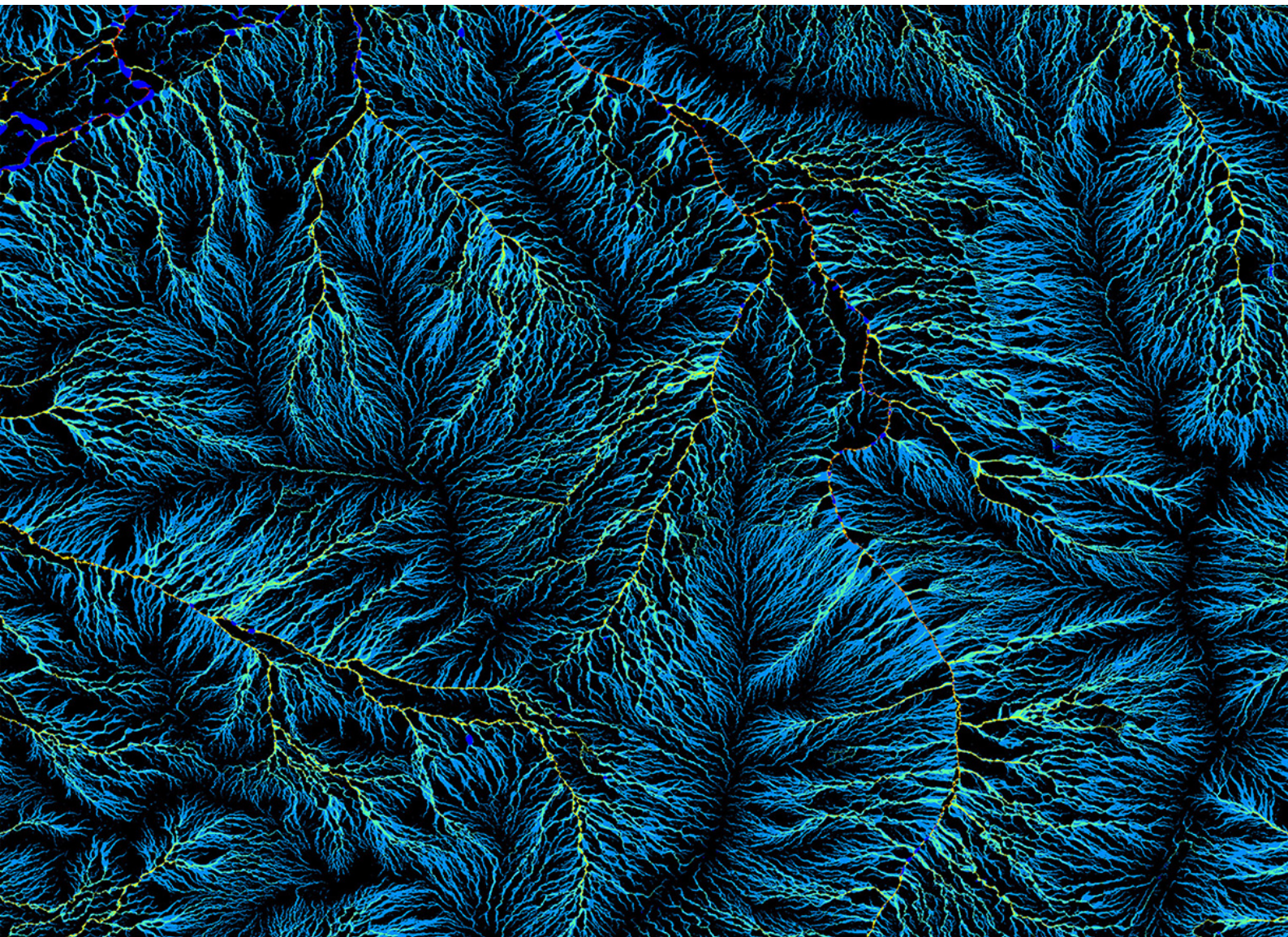

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Executive summary

In 2017, CSIRO in collaboration with AZTI Tecnalia (Spain), IRD (France) and CFR (Indonesia) commenced a 3-year collaborative project on population structure of tuna, billfish and sharks of the Indian Ocean funded by the European Union and the consortium partners (PSTBS-IO). The project aimed to describe the population structure and connectivity of priority tuna and tuna-like species within the Indian Ocean, as well as blue and scalloped hammerhead sharks. Genetic analysis of new and archived tissue samples was the primary method, complimented by microchemical analysis of otoliths. The project also aimed to extend collaborative research networks among partners and contribute to technical capacity building in participating coastal states. Sampling was completed between late 2017 and early 2019 with a total of 5,767 tissue samples and 3,010 otoliths collected or made available to the project from partner archives. Of these, 3,610 tissue samples have been genotyped and 689 processed and analysed for otolith microchemistry. The final data coverage for each species across their range within the Indian Ocean varied among the study species and between genetics and microchemistry methods. For genetics, good sample coverage was achieved for the six neritic and tropical tuna species and swordfish; while the coverage for albacore, the two other billfish species and blue shark limited the power of analyses to examine population structure within the Indian Ocean. The restrictions associated with CITES listing of scalloped hammerhead precluded useful coverage of this species in the project. The sample coverage for otolith microchemistry was often less complete for each species than for genetics due to the logistic difficulty in obtaining otoliths, relative to tissue samples, particularly in the case of larger, more valuable adults. Good coverage was achieved for kawakawa and Spanish mackerel and the three tropical tunas, whereas the lack of otolith samples from the south-east Indian Ocean for albacore limited the scope of inferences that could be made based on microchemistry for this species. The project has provided a sound foundation for exploring specific hypotheses on population structure related to stock assessment and management purposes for the majority of the study species and a good foundation for extending the coverage for the remainder. The preliminary results for each species and method were presented to the 2019 meeting of the Scientific Committee and the final, detailed results from the current project have been presented and reviewed by the respective IOTC Working Parties through 2020. Here, we provide a high-level summary of the population structure results (and links to the detailed working papers) and recommendations on necessary next steps to build on the foundations established and momentum generated by this project. These include, that the IOTC support:

i) the review, discussion and consultation with scientists on the results of the project, their limitations and utility for stock assessment and management purposes, with a view to identifying priorities to be considered by the SC and Commission;
ii) convening a workshop of relevant experts to provide advice on the use of results for the short and medium term, relative to the priorities identified in i); and
iii) development of a targeted biological sample collection program, including tissue and otolith collection for future population structure studies, based on i) and ii), that consolidates and extends the networks developed through this and other recent and current initiatives.

1 Introduction

In 2017, CSIRO (Australia) in collaboration with AZTI Tecnalia (Spain), IRD (France) and CFR (Indonesia) commenced a 3-year collaborative project on population structure of tuna, billfish and sharks of the Indian Ocean funded by the European Union and the consortium partners. The aim of the project was to describe the population structure and connectivity of priority tuna and tuna-like species within the Indian Ocean (and adjacent Pacific and Atlantic waters, as appropriate), as well as blue and scalloped hammerhead sharks. Genetic analyses of new and archived tissue samples was the primary method, complimented by microchemical analysis of otoliths. The project also aimed to develop and extend collaborative research networks among partners and contribute to technical capacity building in participating coastal states. Sampling was completed between late 2017 and early 2019 with a total of 5,767 tissue samples and 3,010 otoliths collected or made available to the project from partner archives. Of these, 3,610 tissue samples have been genotyped and 689 processed and analysed for otolith microchemistry following sample selection and quality control protocols. The final data coverage for each species across their range within the Indian Ocean varied among the study species and between genetics and microchemistry methods. For genetics very good-good sample coverage was achieved for the six neritic and tropical tuna species and swordfish; while the coverage for albacore, the two other billfish species and blue shark limited the power of analyses to examine population structure within the Indian Ocean. The restrictions associated with international transports of samples of scalloped hammerhead following CITES listing precluded useful coverage of this species in the project. The sample coverage for otolith microchemistry was often less complete for each species than for genetics due to the additional logistic difficulty in obtaining otoliths, relative to tissue samples, particularly in the case of larger, more valuable adults. Good coverage was achieved for kawakawa and Spanish mackerel and the three tropical tunas, whereas the lack of otolith samples from the south-east Indian Ocean for albacore limited the scope of inferences that could be made based on microchemistry for this species. The project has provided a sound foundation for exploring specific hypotheses for population structure related to stock assessment and management purposes for the majority of the study species. The preliminary results for each species and method were presented to the 2019 meeting of the Scientific Committee and the final, detailed results from the current project have been presented and reviewed by the respective IOTC Working Parties through 2020. Here, we provide a high-level summary of the population structure results (and links to the detailed working papers) and recommendations on necessary next steps to build on the foundations established and momentum generated by this project.

2 Project reporting and Engagement

2.1 Activities completed to date

A summary of the reporting and engagement with IOTC Scientific Committee for the project is provided in Table 1. The first main reporting of results was the draft final report to the Scientific Committee at its last meeting (2019) (Davies et al., 2019). These results were preliminary, with additional analyses and quality control required for most species. The original project schedule included wider engagement with the IOTC working parties and coastal states at this point to provide additional input and interpretation of the results. Unfortunately, this was not logistically possible in the first quarter of 2020, due to the developing COVID-19 situation at that time. It was possible to hold a smaller technical workshop of a subset of the project team to review the results of all species available at that time and finalise the plan for reporting and wider engagement with the IOTC scientific community. The aim of this engagement strategy was review of the results and initial interpretation included in the final report to the IOTC secretariat (Davies et al., 2020) and to initiate a discussion on their utility for stock assessment and management purposes. Again, the original intent was for working papers to be presented to each of the relevant working party meetings through 2020 and for project team members to attend the meetings in person to provide time to discuss the details of the results and their preliminary interpretation. The COVID-19 situation has not allowed this to occur and, as a result the level of discussion and substantive input from the working parties has been limited by the E-format of the meetings in 2020.

Notwithstanding this, ten working papers have been submitted and presented to the Working Parties on NT, EB, BF, and TT and two are complete and scheduled for 2021 meetings (WPTmT) (see Table 1) providing detailed results for each species and method (population genetics or otolith microchemistry) and some valuable feedback has been provided by the Working Parties. This feedback and, in some cases, additional analysis will be taken into account in the peer review publications of the work, which is scheduled for 2020-2021. The peer review publication process will provide an additional level of technical review and input to consideration of the utility of the results in the stock assessment and management processes of the IOTC. To further this discussion and learning from the experience of this project implementation, we have compiled a summary of the considerations that we think need to be taken into account in the design and interpretation of otolith microchemistry studies for large highly migratory tunas and billfish (Attachment 1).

2.2 Further engagement on interpretation and use of population structure results

Given the technical complexity of the population structure results and their interpretation for stock assessment and management purposes, and the constraints on genuine technical discussion and review imposed by the COVID-19 situation, we strongly recommend that the IOTC support additional engagement with the scientific committee and commission on the interpretation of the final results from the project to ensure the maximum benefit is obtained from this substantial investment and that the results are interpreted and used appropriately. We suggest two

mechanisms for this. First, through the relevant Working Parties, time is allocated in future meetings (ideally, face to face, or perhaps dedicated webinar workshops) to review results in detail and prioritise next steps for each species. Second, following the completion of the more detailed review by the working parties, the Scientific Committee convene an technical workshop, with invited experts with relevant expertise in stock structure, stock assessment and management, to review the outcomes of the Working Parties and provide advice to the SC and Commission on i) the appropriate use of the current results, ii) short and medium term priorities for future work.

2.3 Summary of population structure results

As noted above, the detailed results for each species have been reported in working papers and presentations to the relevant working parties (Table 1). A high-level summary of these results, in terms of the sample coverage, number of potential groups identified (within the sampling range in the Indian Ocean; see Figure 1) and the relative strength of the inference is provided in Table 2.

In summary, the results from the project indicate:

i) That, with the exception of blue shark, striped marlin and Indo-Pacific sailfish, the inter-ocean comparisons for other species are consistent with the Indian Ocean being considered a closed unit for stock assessment and management purposes;

ii) There is strong evidence of genetic partitioning for longtail tuna and Spanish mackerel, with multiple groups identified within the northern and eastern parts of their Indian Ocean range sampled for the project;

iii) There was a common pattern of genetic differentiation between samples from locations north and south of the equator for the three neritic species, skipjack and yellowfin tuna and swordfish. This suggests population structure north and south of the equator for these species.

iv) The strength of inferences based on microchemistry were compromised for a number of species by lack of samples from key locations, wide range of age-classes in the samples, or lack of contrast in the core signature used for identifying potential spawning ground signatures.

v) While the sampling coverage for many species was sufficient to provide substantive results and construct useful hypotheses on population structure within the Indian Ocean, the results are limited to varying degrees by incomplete spatial coverage of the species range in the Indian Ocean, small sample sizes in some locations and/or potential for temporal confounding in timing of sampling.

vi) Further, targeted, multi-year sampling that builds on the collaborative foundations established by this project will be required to make specific recommendations on the implications of these initial results for stock assessment and management.

Below we elaborate on the results for each of the species. Readers interested in the more detailed technical aspects of the results and/or methods for each of the aspects are referred to the working papers to the respective Working Parties listed in Table 1.

2.4 Neritic Tuna

The strongest genetic differentiation and greatest extent of genetic partitioning was evident for two of the neritic species, with three genetic groups within the Indian Ocean identified for longtail tuna and four for Spanish mackerel (Table 2, Feutry et al., 2020). This genetic partitioning also corresponded closely with the sample locations. The strength of the differentiation provides a very clear example of the power of the SNP markers to identify population structure. The results from the otolith microchemistry for these two neritic species were not as clear, partly due to the reduced sample coverage relative to the genetics, but they could be interpreted as being generally consistent with the pattern seen in the genetics. Previous studies of population structure on these species have also demonstrated the utility of microchemistry for detecting finer-scale population structure (e.g. Buckworth et al. 2007). However, as noted in the detailed micro-chemistry results for each of these species, care needs to be taken in the spatial interpretation of these results given the differences in the time of the sampling and the different age classes of fish in the different locations.

Interestingly, the level of genetic differentiation evident for longtail tuna and Spanish mackerel was not apparent for kawakawa. There was evidence of genetic structure between locations north and south of the equator, as noted above, but not the finer scale structure evident for the other two neritic species. This is despite the fact that the sample coverage for genetics for kawakawa was the best of all of the neritics and extended into pelagic environments in the central and western-central Indian Ocean outside, the sampled range of the other two species, where most of the samples came from purse-seine catches associated with targeted fishing for skipjack. The otolith core microchemistry for kawakawa did not provide additional evidence for population structure from a spawning ground perspective. The results of the otolith edge microchemistry did indicate some structure, similar to that for longtail tuna, but again, the extent to which this reflected spatial population structure versus temporal differences in sampling among sampling locations cannot be determined with the available samples (Davies et al., 2020).

The clear genetic differentiation among locations for long-tail tuna and Spanish mackerel is strong evidence for separate stocks within the sampling range covered by this project. Despite the best efforts of the project team, it does not cover the full range of these species within the Indian Ocean. The results for these two neritic species provide a compelling case for the design and implementation of future studies to: i) extend the sampling coverage to the full range of these species within the Indian Ocean; ii) increase sample sizes and temporal coverage (i.e. establish sampling capacity for multi-year program); and, iii) extend to the other identified priority neritic species (bullet and frigate tuna and Indo-Pacific king mackerel) to provide comprehensive description of population structure of these important neritic species for stock assessment and management purposes within the Indian Ocean.

2.5 Tropical Tuna

There was evidence of population structure within the Indian Ocean for skipjack (Rodríguez-Ezpeleta et al., 2020) and yellowfin tuna (Grewe et al., 2020a), but not for bigeye tuna (Díaz-Arce, et al., 2020). Analysis of out-locations indicated low genetic connectivity with the Atlantic and

Pacific Oceans for all three species, which supports the IO populations being managed independently of populations in neighbouring oceans.

Within the Indian Ocean there was evidence of genetic population structure between locations north and south of the equator for both skipjack and yellowfin, but no evidence of genetic differentiation within the Indian Ocean locations for bigeye.

For yellowfin, there was evidence of additional genetic structure within the locations north of the equator, although the strength of this inference was weaker than that supporting the north-south differentiation (Grewe et al 2020a). The results for otolith core analysis for yellowfin Artetxe-Arrate, et al 2020) also demonstrated a distinct signature for one northern location (Pakistan) and relative to the core signatures of young of the year from southern locations. While this micro-chemistry result is not unequivocal, it is consistent with the pattern observed from the genetics for yellowfin tuna (and other species), suggesting that the NW/Arabian sea samples are substantially different areas south of the equator.

In the case of skipjack, the evidence for potential structure was more complicated (Rodríguez-Ezpeleta et al., 2020), in that: the genetic groupings identified by the analysis did not align uniquely with individual locations/groups of locations and there was also a high number of closely related individuals (kin-pairs) identified in the samples from some locations. Hence, these initial results should be interpreted with caution and additional analysis of the existing data are continuing. In addition, collection of additional samples is underway which are expected to be analysed in the coming year. The analysis of otolith cores of young of the year skipjack did not indicate any significant differences in stable isotopes or micro-elements among the three (west, central and east) locations analysed.

2.6 Albacore

Genetic analysis of out-locations indicated that there was genetic differentiation between the Indian Ocean and the Atlantic and Pacific Oceans (Davies et al., 2020) for albacore. In particular, samples from the south-east Atlantic that were sampled from the Atlantic coast of South Africa, grouped with the north Atlantic samples. Samples were available from only two locations within the Indian Ocean (south-west and central-east) and there was no evidence of genetic structure between them. Availability of samples from sufficient locations (and narrow range of size/age classes) for otolith microchemistry also limited our ability to make strong inferences about population structure within the Indian Ocean (Davies et al., 2020). In future, development of structured sampling programs with key distant water fishing fleets targeting albacore, to obtain samples from the central-southern and south-eastern Indian Ocean, and Indonesia, for samples from the spawning grounds south of Java, will be important to improve the understanding of population structure of albacore within the Indian Ocean.

2.7 Billfish

Sampling coverage or both striped marlin and sailfish was insufficient to unequivocally conclude whether there was evidence of gene flow being sufficiently restricted for the Indian Ocean to be considered effectively isolated from the Atlantic and/or Pacific Oceans for fisheries management purposes (Grewe et al 2020b). In the case of striped marlin, there was also insufficient samples to

explore structure within Indian Ocean. There was no evidence of genetic structure between the two Indian Ocean locations (western-central and north-east) for Ind-Pacific sailfish. Clearly, further targeted sampling for these two species is required to provide more robust and informative analysis.

Analysis of Indian and Pacific Ocean (Tasman and Coral Seas) samples of swordfish provided evidence of sufficiently restricted geneflow (Grewe et al 2020b) for the Indian and south-west Pacific Ocean populations to be considered separate for management purposes. Previous studies have demonstrated the level of connectivity between the Atlantic and Indian Oceans is also sufficiently restricted for the Indian Ocean to also be considered separate from the Atlantic Ocean (see discussion in Grewe et al., 2020b). The analysis of within Indian Ocean structure suggests subtle population structure with potentially two, or more, genetically differentiated groups north and south of the equator. The two southern (south-west and south-east) locations are genetically most similar to the eastern-central location, while the north-east, north-central and west-central locations group together. Additional temporally stratified sampling of adults on known spawning and feeding grounds and extension of sampling into the north-west and north-east extremities of the range in the Indian Ocean are required to provide a robust and comprehensive assessment of the population structure for stock assessment and management purposes (Grewe et al., 2020b).

The otolith micro-chemistry analysis were also indicative of structure within the Indian Ocean, with three putative spawning origins identified (Darnaude et al., 2020). Caution is require in their interpretation, however, due to the potentially confounding by cohort effects and seasonal and inter-annual variation in environmental conditions. Rigorous spatial and temporal stratification of sampling is required to improve the strength of inferences from otolith microchemistry. This proved too challenging to achieve in this study, particularly for billfish (Davies et al., 2020).

2.8 Blue shark

The combination of archival samples from consortium partners and new samples from three Indian Ocean locations allowed for a global analysis for blue shark (Nikolic et al., 2020). This revealed two main genetic clusters for blue shark globally: the northern Atlantic Ocean region, including the Mediterranean Sea, and the Indo-Pacific region. This is the first time that genetic structure has been demonstrated for blue shark. Given the relatively low number of sample locations (and sample sizes) achieved in the Indian Ocean for this project, the development of a targeted, coordinate sampling program that includes multiple Indian Ocean locations as well as south-east Atlantic and south-west Pacific sampling should be a priority.

2.9 Conclusions and Recommendations

The scope and aims for this project were ambitious and not all have been achieved. It has, however, provided evidence of population structure within the Indian Ocean for a number of the key target species (2 species of neritic tuna, 2 species of tropical tuna and one billfish) and provided the first evidence of structure in blue sharks at the global level.

There are a number of important considerations in considering the results from this project.

The project has provided a sound foundation for exploring hypotheses for population structure for many of the study species. For some species, such as albacore, improvements in sample coverage, or additional samples from locations with low sample sizes, are required before substantive interpretations and conclusions about population structure across the Indian Ocean can be made. It will be important, therefore, that the samples and data collected through this project are appropriately archived and curated to ensure they are available for use in future studies. The project partners will work with the IOTC secretariat and Scientific Committee to finalise arrangements for archiving, access and management of the samples and the data arising from the project, so that this foundation data set is available to build the understanding of population structure of these species into the future.

In the case of the population genetic analysis, failure to detect population structure for a particular species with a particular set of samples and method, does not prove absence population structure that may be important from an assessment or monitoring perspective. It is possible that more comprehensive sampling across the range of the species than was possible in this project and the application of new methods may provide additional information on the structure and connectivity of these populations, which is not evident in the current results.

In this context, the results of this project and their interpretation should be seen as working hypotheses on population structure. They provide a solid foundation for initial deliberations by the IOTC Working Parties and Scientific Committee on their implications for assessment and management of the stocks under IOTC purview, and design of future targeted studies, for individual or multiple species, to test their validity and further refine our understanding and inform stock assessment and management of these important fisheries.

Given the importance of the results to future assessment and management of IOTC fisheries, we recommend that the IOTC support:

i) the review, discussion and consultation with scientists on the results of the project, their limitations and utility for stock assessment and management purposes, with a view to identifying priorities to be considered by the SC and Commission;

ii) convening a workshop of relevant experts to provide advice on the use of results for the short and medium term, relative to the priorities identified in i); and

iii) development of a targeted biological sample collection program, including tissue and otolith collection for future population structure studies, based on i) and ii), that consolidates and extends the networks developed through this and other recent and current initiatives.

Figures

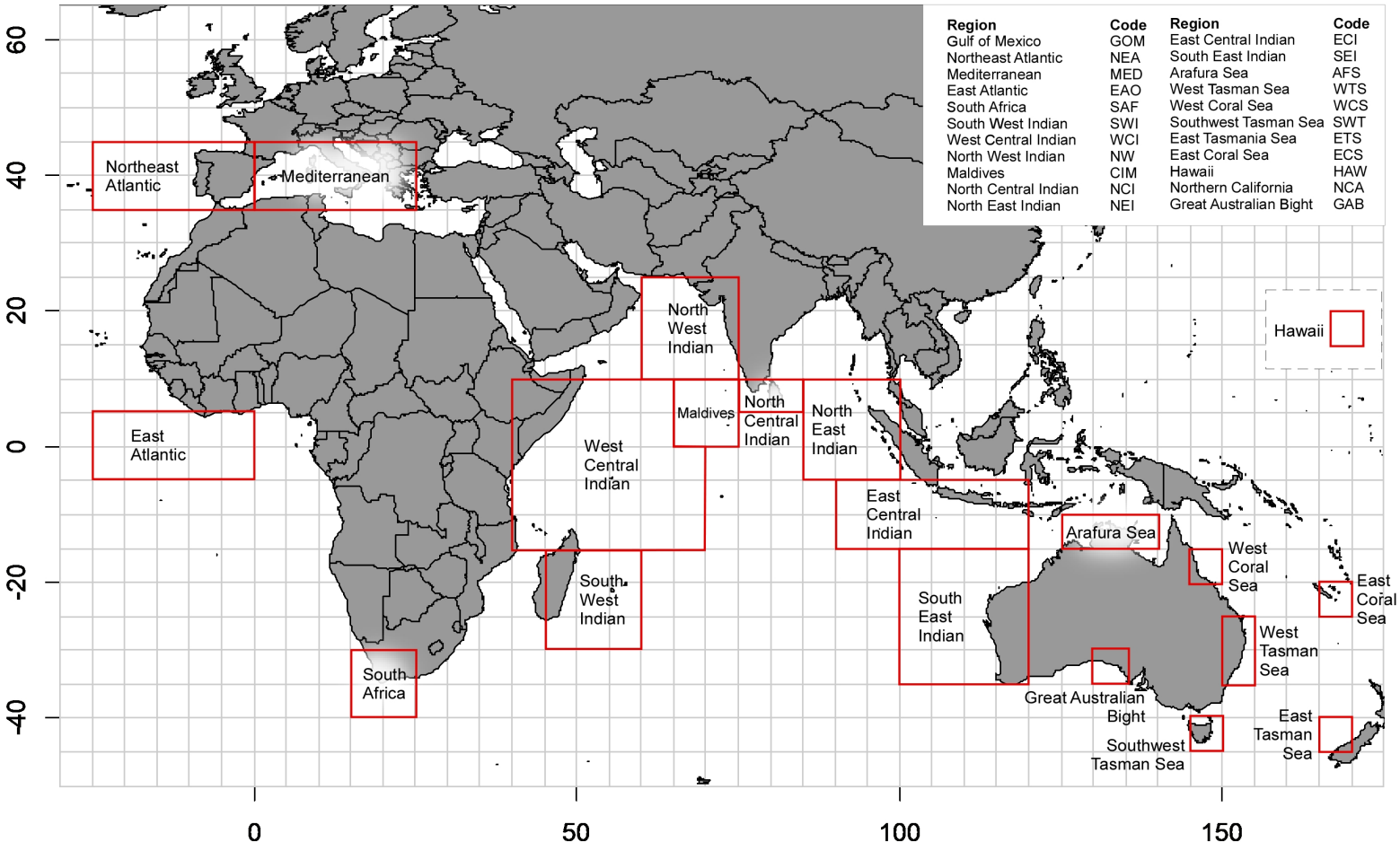

Figure 1. Distribution of sampling locations for both rounds of sampling across Indian Ocean and outlier locations in the Pacific and Atlantic Oceans. Note: this includes locations for active sampling as well as locations for samples provided from earlier studies (see text for details).

Tables

Table 1. Summary of reports and IOTC Working Party and Scientific Committee papers resulting from the Population structure of IOTC species and sharks of interest in the Indian Ocean (PSTBS-IO) project. LOT = longtail tuna, KAW = Kawakawa, COM = Narrow-barred Spanish mackerel, SFA = Indo-Pacific Sailfish, MLS = Striped Marlin, SWO = swordfish, BSH = blue shark, SKJ = skipjack, BET = bigeye tuna, YFT = yellowfin tuna, ALB = albacore tuna

Species	Report title	Year	Authors	Reference	URL
Project reporting					
All	Literature Search: Summary Report	2017	Davies, C, Farley J, Sharples R.	NA	NA
All	Progress Report to IOTC (x7)	2018-2019	Davies, C.	NA	NA
All	2019 Annual Report to IOTC	2019	Davies C, Marsac F, Murua H, Fraile I, Famhi Z, et al.	NA	NA
All	Final Report to IOTC	2020	Davies C, Marsac F, Murua H, Fraile I, Famhi Z, et al.	NA	NA
Working Papers to Working Parties and Scientific Committee					
All	Population structure of IOTC species and sharks of interest in the Indian Ocean	2017	Davies C, Murua, H. Marsac F, Fahmi Z.	IOTC-2017-SC20-INF0-08	https://iotc.org/documents/population-structure-iotc-species-and-sharks-interest-indian-ocean
All	Population Structure of IOTC species and sharks of interest in the Indian Ocean	2019	Davies C, Marsac F, Murua H, Fraile I, Fahmi Z, et al.	IOTC-2019-SC22-INF05_Rev1	https://iotc.org/documents/SC/22/INF05
LOT, KAW, COM	Genetic population structure of neritic tunas in the Indian Ocean from the PSTBS-IO Project	2020	Feutry P, Foster S, Grewe P, Aulich J, Lansdell M, et al.	IOTC-2020-WPNT10-10	https://iotc.org/documents/WPNT/10/10
SFA, MLS, SWO	Genetic population structure of sailfish, striped marlin, and swordfish in the Indian Ocean from the PSTBS-IO Project	2020	Grewe P, Feutry P Foster S, Aulich J, Lansdell M et al.	IOTC-2020-WPB18-09	https://iotc.org/documents/WPB/18/09
SWO	Otolith microchemistry suggests probable population structuring in the Indian Ocean for the broadbill swordfish	2020	Darnaude A, Labonne M, Petit C, Médieu A, Pernak M et al.	IOTC-2020-WPB18-10_Rev1	https://iotc.org/documents/WPB/18/10

BSH	Genome scans discriminate independent populations of the blue shark Prionace glauca	2020	Nikolic N, Devloo-Delva F, Bailleul D, Noskova E, Rougeux C, et al.	IOTC-2020-WPEB16-14	https://iotc.org/documents/WPEB/16/14
SKJ	Investigating early stages of skipjack tuna (*Katsuwonus pelamis*) in the Indian Ocean using otolith chemistry	2020	Artetxe-Arrate I, Fraile I, Rodriguez-Ezpeleta N, Farley J, Darnaude AM, et al.	IOTC-2020-WPTT22(AS)-05_Rev1	https://iotc.org/documents/WPTT/2202/05
SKJ	Co-occurrence of genetically isolated groups of skipjack tuna (Katsuwonus pelamis) within the Indian Ocean	2020	Rodriguez-Ezpeleta N, Artetxe-Arrate I, Mendibil I, Diaz-Arce N, Krug I, et al.	IOTC-2020-WPTT22(AS)-07	https://iotc.org/documents/WPTT/2202/07
YFT	Otolith δ18O as a tracer of yellowfin tuna (*Thunnus albacares)* nursery origin in the Indian Ocean	2020	Artetxe-Arrate I, Fraile I, Farley J, Clear N, Darnaude AM, et al.	IOTC-2020-WPTT22(AS)-06_Rev1	https://iotc.org/documents/WPTT/2202/06
YFT	Genetic population connectivity of yellowfin tuna in the Indian Ocean from the PSTBS-IO Project	2020	Grewe P, Feutry P, Foster S, Aulich J, Lansdell M, et al.	OTC-2020-WPTT22(AS)-12_Rev1	https://iotc.org/documents/WPTT/2202/12
BET	Investigating population structure of bigeye tuna in the Indian Ocean using otolith chemistry	2020	Clear N, Eveson P, Darnaude AM, Labonne M, Artetxe-Arrate I, et al.	IOTC-2020-WPTT22(AS)-11	https://iotc.org/documents/WPTT/2202/11
BET	Evidence of connectivity of bigeye tuna (*Thunnus obesus*) throughout the Indian Ocean inferred from genome-wide genetic markers	2020	Diaz-Arce N, Grewe P, Krug I, Artetxe-Arrate I, Ruiz J, et al.	IOTC-2020-WPTT22(AS)-16	https://iotc.org/documents/WPTT/2202/16
ALB	Genetic analysis of albacore tuna (*Thunnus alalonga*) population structure (draft title)	2021	Nikolic N, et al.	IOTC-2021-WPTmT08	NA
ALB	Otolith microchemistry analysis of population structure – albacore (*Thunnus alalunga*) (draft title)	2021	Labonne M, et al.	IOTC-2021-WPTmT08	NA

Table 2: Summary of sampling coverage, population structure inferences and future work priorities by species and method for the PSTBS-IO project. *G*= NGS genetics, M= otolith microchemistry. *N* = number of samples used in final analysis; *note for many species there are additional samples available that have not been analysed and some samples were excluded from the final analysis following quality control checks. *% IO Range*: the approximate proportion of the species range in the IO covered by sampling. *Potential No. Groups* = is the potential number of "groups" within the range covered by the sampling within the IO and does not include the sampling locations outside the Indian Ocean. In most cases, **this is not definitive** and requires further work to consolidate these initial hypotheses and extend the sampling to the full range of the species within the Indian Ocean. *Strength of Evidence*: is a qualitative measure based on the sample coverage, the strength of the results of the statistical analysis and the expert judgement of the project team. *Information required*: specific recommendations for each species on the additional sampling/analysis required to test the preliminary hypothesis and/or provide the sample coverage required for basin scale inferences of population structure. *Relative priority*: project teams assessment of the relative priority of information, given data and results available, value of information in the current IOTC context, logistic difficulty/cost of information.

Species	**Method**	**Sampling Coverage***			**Potential No. Groups**	**Strength of evidence**	**Information required to consolidate initial results and inform monitoring and management**
		N	No. IO Regions	% IO Range			
LOT	G	221	3	<50	3	Strong	Multi-year sampling <3mth old fish/spawning adults, across full IO range, stratified by monsoon, same year. Priority to is to extend coverage to include NW and SE of the range, including finer scale sampling between current locations and more uniform size/age range of <3mth old fish/spawning adults.
	M	62	3	50	2	Weak	Multi-year sampling <3mth old fish, across full IO range, stratified by monsoon, in the same year. Priority would be to sample narrow size range of same year class in each sampling window.
KAW	G	308	7	75	2	Strong	Multi-year sampling <3mth old fish/spawning adults across full IO range, stratified by monsoon. Priority would be extension into far NW and NE.
	M	85	4	50	2-3	Weak	Multi-year sampling <3mth old fish, across full IO range, stratified by monsoon, same year. Lack of significant difference in core signature suggests there may not be sufficient contrast in environmental signal to detect spawning ground signature.
COM	G	189	5	50	4	Strong	Multi-year sampling extending to W, NW and SE extremes of range. Finer scale sampling between identified groups and more uniform size/age range of <3mth old fish/spawning adults.
	M	80	4	50	3	Weak	Multi-year sampling <3mth old fish, across full IO range, stratified by monsoon, same year.
SKJ	G	393	5	75	2	Moderate	Multi-year sampling <2mth old? Fish/larvae, across full IO range, stratified by monsoon, same year.

	M	50	3	60	1	Moderate	Multi-year sampling <2mth old? Fish/larvae, across full IO range, stratified by monsoon, same year.
YFT	G	546	7	85	>2	Strong for N-S; Moderate for >2 in N.	Multi-year sampling <3mth old and spawning fish from known grounds, across full IO range, stratified by monsoon.
	M	180	7	75	>2	Moderate	Multi-year sampling <3mth old and spawning fish from known grounds, across full IO range, stratified by monsoon, same year.
BET	G	472	7	75	1	Moderate	Multi-year sampling of spawning fish <3mth old in E-W and N-S of equator in the E, stratified by monsoon.
	M	101	4	60	>1?	Weak	Multi-year sampling of <3mth old fish, across full IO range, stratified by monsoon, same year.
ALB	G	224	3	50	1?	Weak	Multi-year, temporally stratified sampling of adult fish on spawning and feeding grounds and of juveniles in SW. Confirmation of presence/capacity to sample YoY fish in SE.
	M	80	2	50	2-3?	Weak	Multi-year, temporally stratified sampling of YoY in spawning regions in over multiple years.
SWO	G	309	6	75	2	Moderate	Multi-year temporally stratified, sampling of adult fish/larvae on spawning grounds and juveniles/adults on feeding grounds, in particular the identified spawning regions north and south of the equator.
	M	70	3	60	2-3	Moderate	Multi-year, temporally stratified sampling of adult fish on spawning and feeding grounds and of YoY on spawning grounds. Priority is Spawning adults/larvae from identified spawning grounds.
MLS	G	20	1	50	1	Weak	Multi-year, temporally stratified, sampling of adult fish/larvae on spawning and juveniles/adults on feeding grounds. Increased engagement with more coastal fisheries to obtain sufficient samples.
SLA	G	65	2	50	1	Moderate	Extended, temporally stratified, sampling of adult fish/larvae on spawning and juveniles/adults on feeding grounds. Increased engagement with more coastal fisheries to obtain sufficient samples.
BSH	G	364	4	60	1	Moderate	Increased multi-year coverage with IO and extended cooperation with DWFN. Requires coordinated sampling with, at least, SE Atlantic and SW Pacific to address connectivity.
SPL	G	-	-	-	-	-	Future sampling and analysis can be conducted under appropriate CITES arrangements.

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Attachment 1: Considerations on the interpretation of otolith micro-chemistry results for tuna and billfish and for future research activities.

The key underlying assumption of this work is that an environmental signal can be identified in otoliths and used to differentiate groups of fish. However, otolith composition is influenced by both intrinsic and extrinsic factors that may confound the environmental signal. For example, ontological changes influence otolith composition according to the life stage of the fish and, subsequently, elemental and stable isotopes signals can vary in the otoliths of larvae, juveniles and adults occupying the same environment. The complex nature of otolith deposition is evident in the results of the PSTBS-IO and some general findings and interpretations are below:

- Where no differences are detected in otolith microchemistry from fish of the same age and life stage, we infer either:
  1. they occupied the same area, or
  2. they occupied different areas with similar water chemistry.

- Where significant differences are detected in otolith microchemistry from fish of the same age and life stage, we infer either:
  1. they occupied different areas, or
  2. they occupied the same area but were caught at different times of the year with different water chemistry e.g. during the southwest/summer and northeast/winter monsoons.

- Where significant differences are detected in otolith microchemistry at different life stages (e.g. larvae versus juvenile), this may indicate:
  1. fish migrating, or larvae drifting, from one area to another with different water chemistry between the two life stages, or
  2. residency in the same area but ontological influences producing a different otolith composition, or
  3. residency in the same area but oceanography having changed during the time between the 2 life stages.

The uncertainties in the above situations can be addressed by:

- Constraining the sampling dates when comparing groups of fish, i.e. fish are analysed by season and by year.
- Acquiring and incorporating more knowledge of seasonal and annual variation in oceanographic conditions to understand how certain elements and stable isotopes vary.
- Improving and incorporating more knowledge of the biology and behavior of species, particularly in relation to spawning areas. For example, yellowfin and bigeye tuna undergo extensive migrations between their spawning areas in equatorial waters and their feeding grounds in higher latitudes. This pattern is especially true for adult bigeye, which are distributed deeper and at more temperate latitudes hence bigeye tuna are more likely to move towards the colder and richer subtropical areas, after leaving the spawning areas, rather than dispersing across tropical waters. Skipjack, however, undertake predominantly diffusion-style movements, being distributed across tropical areas, which are suitable for them to spawn and to feed. They reach maturation early; spawning occurs all year round and is generally opportunistic. Albacore on the other hand spawn in specific areas in the tropics of each

ocean and have a complex life history in which juveniles and adults separate geographically but can occupy the same areas at different times.

- Understanding spawning behavior and patterns of larval dispersal. Useful information could be supplied from biophysical and particle dispersal models, including predicted passive drift trajectories based on langragian particle simulations (see Nikolic et al 2020).
- Combining knowledge of spawning and oceanography, specifically, identifying the location and timing of spawning, and identifying which monsoonal events and oceanographic features, including sub mesoscale circulations, mesoscale eddies and major currents coincide with spawning and how they affect spawning events. For example, peak spawning of bigeye tuna in the Indian Ocean has been reported during the winter monsoon in the west, but during both the winter and summer monsoons in the east. Sub-mesoscale circulations influenced by the monsoonal patterns can minimize long-distance larval dispersal and hence retain larvae near their spawning area.
- Using fisheries data, including data from FADS, to provide information on catches at different life stages.

Based on the above, recommendations for future studies of otolith microchemistry, species biology and oceanography in the Indian Ocean include:

Sampling

- Target individuals from the same age classes and hatched under the same monsoonal regime to better account for interannual variability and intra-annual (seasonal) variability among fish from the same area. For accurate ages, comprehensive growth and age validation studies are needed that include small sizes because current studies extrapolate the length-age relationship to smaller fish rather than incorporate direct ageing data.
- The seasonal variation in the Indian Ocean may strongly influence otolith chemical composition so, similar to above, further studies should consider temporal stratification of sampling (intra and inter-annual), so that seasonal differences in oceanography can be partitioned from potential regional differences in stock structure.
- Analyse YOY that are as small as possible to avoid potential movements between hatching and sampling, from known spawning areas collected over several years/seasons to set up a baseline for matching otolith cores from older fish collected in other areas of the Indian Ocean. Current knowledge of spawning areas of the IOTC species is sparse, so we encourage further research in areas where spawning adults are caught, in order to identify more potential spawning grounds. The degree of mixing and connectivity between different areas could be assessed by targeting adults from different fishing areas. Targeting adults in spawning areas at spawning season would allow investigation of fidelity to the spawning grounds.
- Sample the maximum numbers of contributing sources as possible. There are areas considered to be potential spawning grounds which were not sampled in the current study (e.g. Pakistan, Sri Lanka, South Java) that could be included in future research.
- Collecting monthly water samples in the main nursery areas throughout year may help better understand seasonal variations of elemental and isotopic composition of seawater.

Species biology

- Launch collaborative research projects to sample and/or use currently available biological material to analyse spawning areas to investigate spawning areas of main species in the Indian Ocean. Improved knowledge to be acquired about spawning areas from *IOTC-FAO Development and implementation of a*

sampling scheme to support the collection of biological samples and conduct analysis on these samples to provide improved estimates of age, growth and reproduction of tropical tunas, swordfish, and blue sharks for the Indian Ocean Tuna Commission (IOTC).

Otolith microchemistry

- Make an informed choice of elements/isotopes. There is increasing evidence that some elements simply add endogenous variability to the dataset. Stable isotope data are useful to discriminate among the most distant areas latitudinally, E-W, but additional elements are needed to discriminate intermediate (central, or NE, SE, NW, SW) areas. Furthermore, carbon and oxygen stable isotopes mainly reflect variation in the environment, however they may be not be sufficient to discriminate among different groups of fish in the Indian Ocean, so should be combined with trace element data.
- Refine methodological procedures where required. Some tunas and billfish have very small otoliths that can be easily broken during preparation and analysis, so a challenge for further studies will be to modify the preparation to ensure more samples remain intact.
- Integrate otolith microchemistry results with other sources of information. Depending on the season, the Indian Ocean might be quite homogeneous in terms of oceanography, such that the use of otolith microchemistry alone may not provide enough information to elucidate stock structure. Combining with other techniques, e.g. genetics, parasitic data, biophysical models, will increase the power to resolve stock structure patterns.
- Select appropriate statistical analyses and interpret results judiciously. Given otolith chemistry can vary with ontological, spatial and temporal factors, plus large individual variation can occur between fish of the same age residing in the same area at the same time, careful consideration must be given to how the data are analysed and the results interpreted. For example, large individual variability may mask spatial differences; conversely, ontological and temporal differences may incorrectly be interpreted as spatial differences unless the sample design and chosen statistical analyses are such that these confounding factors can be accounted for.

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