SCIENTIFIC COMMITTEE
FOURTEENTH REGULAR SESSION

Busan, Republic of Korea
8-16 August 2018

Connectivity of tuna and billfish species targeted by the Australian Eastern Tuna and Billfish Fishery with the broader Western Pacific Ocean

WCPFC-SC14-2018/ SA-IP-04

Evans K, P. Grewe, R. Gunasekera and M. Lansdell[1]

[1] CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart Tasmania 7001, Australia

Connectivity of tuna and billfish species targeted by the Australian Eastern Tuna and Billfish Fishery with the broader Western Pacific Ocean.

Karen Evans, Peter Grewe, Rasanthi Gunasekera, Matt Lansdell.

CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart Tasmania 7001, Australia

Abstract

Australia's Eastern Tuna and Billfish Fishery (ETBF) harvests stocks of tunas and billfish that are shared across a range of fisheries in the adjacent Pacific Ocean and are managed under the Western and Central Pacific Fisheries Commission (WCPFC). Management of these fisheries is complex due to the cross-jurisdictional nature of the stocks and associated management at domestic and regional scales. Current assessments conducted by the WCPFC assume that these species comprise single panmictic stock units present either throughout the WCPFC area or across the Southern Hemisphere portion of the region. Biological information on growth rates and reproduction, movement data derived from tagging studies and spatial and temporal variability in catches of other tuna and billfish species however, suggest that populations throughout the WCPFC region may be structured. Recent investigations into the stock structure of yellowfin tuna using next generation sequencing (NGS) methods have identified the presence of previously undescribed structure within populations across the Western and Central Pacific Ocean. These observations challenge current assumptions of stock structure and suggest that they may not accurately reflect the biology of commercially important tuna and billfish species throughout the region.

A three-year project funded through the Fisheries Research Development Corporation on behalf of the Australian Government and the CSIRO commenced in July 2016. The goal of this project is to use next generation sequencing technology to improve understanding of the population structure of five species targeted in the ETBF (albacore, bigeye and yellowfin tunas, broadbill swordfish and striped marlin) and examine their connectivity with the broader WCPFC region. This paper provides an update on project progress to date and describes plans for the final year of the project. It also provides suggestions for future planning around the WCPFC Tissue Bank in light of processing of samples from the Tissue Bank as part of this project.

Background

Australia's Eastern Tuna and Billfish Fishery (ETBF) operates in waters off on the east coast of Australia and catches a number of pelagic species including yellowfin, bigeye and albacore tuna, swordfish and striped marlin. Populations of these species are known to extend well beyond the Australian Exclusive Economic Zone (EEZ) and are considered to form part of at least a wider Western Pacific Ocean (WPO) population, although specifics on connectivity between various regions is still a major source of uncertainty. Populations are currently assessed as a single inter-connected stock distributed across the wider western and central Pacific Ocean or South Pacific Ocean and are managed at the international level under the auspices of the Western and Central Pacific Fisheries Commission (WCPFC).

Although populations are assessed as single interconnected stocks, biological information on growth rates and reproduction, movement data derived from tagging studies and spatial and temporal variability in catches of these species suggest that there is likely to be some structure to stocks throughout the WCPFC region. More recently, both traditional and next generation high throughput

genotyping methods have provided evidence of population structure in yellowfin tuna across the Pacific (e.g. Aguilar et al. 2015; Grewe et al. 2015) and provide some support to the hypothesis that yellowfin tuna fished by Australia's tuna fisheries may be a localised stock within the Coral and Tasman Sea region. If yellowfin tuna or the other principal species occurring in the ETBF do comprise localised stocks, this has implications for current consideration of species within stock assessments conducted by the WCPFC (that currently consider most species to comprise a single stock) and associated management of species both within national and regional contexts.

The technical advances of DNA profiling used to investigate the population structure of yellowfin tuna now provide for high throughput sequencing platforms and improved power of population discrimination at much reduced cost. These methods have the potential to test the "single stock" paradigm for highly migratory stocks and provide the technical foundation for global chain of custody and provenance systems necessary to improve accuracy of catch reporting and curb Illegal, Unregulated, and Unreported (IUU) fishing (Grewe et al. 2016). Australia's national research agency, the Commonwealth Scientific and Industrial Research Organisation (CSIRO), has invested in approximately a decade of work in developing a suite of technological advancements including DNA profiling techniques and specialised laboratory processing protocols associated with sample handling, quality control and statistical analysis methods.

Using this technology, a three-year project funded through the Fisheries Research Development Corporation on behalf of the Australian Government and the CSIRO (see Evans et al. 2016; 2017) aims to provide an improved understanding of the population structure for five of the species caught in the ETBF (albacore, bigeye and yellowfin tunas, broadbill swordfish and striped marlin). The project also aims to establish the connectivity of the five species within the broader WCPFC region.

This project builds on previous studies conducted by the CSIRO that have documented genetic structure in yellowfin across three locations in the western, central, and eastern Pacific Ocean and is part of a broader program of work being conducted by CSIRO on the stock structure of pelagic and neritic species across the Indian and Pacific Oceans (Grewe et al. 2016). Outputs from these projects are expected to provide essential information required for the assessment and management of marine species and in particular tuna and billfish species within the two ocean basins.

Methods

Sample collection

Using the output of a spatial assessment of tissue samples for tropical tuna and billfish species held in the WCPFC Tissue Bank and historical samples held by CSIRO, key areas where samples are available for stock structure analyses of yellowfin, bigeye and albacore tunas, broadbill swordfish were identified and an application to access these samples submitted to the WCPFC. Where samples currently held in collections did not meet the experimental design requirements for resolving stock structure, the feasibility of further sampling to resolve spatial gaps and/or inadequate numbers was explored. Within the ETBF, collection of additional samples to those held in CSIRO archives was conducted via sampling of fish during onshore processing. External to the ETBF, collection of samples has been undertaken by project collaborators as part of routine operations. Minimum sample sizes for stock assignment collection of samples aimed to achieve 50 samples from each of two years for each species. The sampling strategy for the project aimed to include three spatially restricted locations, one from the ETBF and two sites within the western Pacific Ocean.

DNA extraction

Biopsies of white muscle were obtained from individuals and approximately 15mg of tissue was subsampled from these biopsies to be used for DNA extractions. Total genomic DNA was isolated using one of two protocols; either a Machery Nagel Nucleo-Mag bead-based DNA isolation kit or a CTAB protocol, a Phenol-Chloroform based method described by Grewe *et al*. (1993). The bead-based extractions were performed on an Eppendorf EP-Motion-5075 robotic liquid handling station. DNA aliquots were shipped to Diversity Array Technologies in Canberra where DNA complexity reduction and library construction was performed prior to sequencing that was used to generate genotype data for each individual.

Genetic sequencing

DArTseq genotyping

The sequencing protocols used incorporated a DArT-Seq proprietary next generation sequencing methodology. DArTseq™ represents a combination of DArT complexity reduction methods and next generation sequencing platforms (for detailed description see Grewe et al., 2015). This represents a new implementation of sequencing complexity with reduced representations and more recent applications of this concept on the next generation sequencing platforms. Similar to DArT methods based on array hybridisations, the technology is optimized for each organism and application by selecting the most appropriate complexity reduction method (both the size of the representation and the fraction of a genome selected for assays). Four methods of complexity reduction were tested in tuna (data not presented) and the PstI-SphI method were also selected for examination of billfish species used in this study. DNA samples were processed in digestion/ligation reactions using a single *Pst*I-compatible adaptor with two different adaptors corresponding to two different Restriction Enzyme (RE) overhangs. The *Pst*I-compatible adapter was designed to include Illumina flow cell attachment sequence, sequencing primer sequence and "staggered", varying length barcode region. The reverse adapter contained a flow cell attachment region and a *Sph*I-compatible overhang sequence.

Only "mixed fragments" (*Pst*I-*Sph*I) were effectively amplified by PCR. PCR conditions consisted of an initial denaturation at 94°C for 1 min followed by 30 cycles of 94°C for 20 sec, 58°C for 30 sec and 72°C for 45 sec, with a final extension step at 72°C for 7 min. After PCR, equimolar amounts of amplification products from each sample of the 96-well microtiter plate were bulked and applied to cBot (Illumina) bridge PCR, followed by sequencing on an Illumina Hiseq2000. The sequencing (single read) was run for 77 cycles.

Sequences generated from each lane were processed using a proprietary DArTseq analytical pipeline (DArT-Soft14 version). In the primary pipeline, the FASTQ files were first processed to filter away poor-quality sequences, applying more stringent selection criteria to the barcode region compared to the rest of the sequence. In that way the assignments of the sequences to specific samples carried in the "barcode split" step was very reliable. Approximately 2,000,000 sequences per barcode/sample were identified and used in marker calling. Finally, identical sequences were collapsed into "fastqcall files". These files were used in the secondary pipeline for DArTseq PL's proprietary SNP and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14). For the current study only co-dominant SNP-DArT markers were used for population analysis.

Progress to date

Historical collections of samples

A spatial assessment and inventory of tissue samples held by CSIRO and in the WCPFC Tuna Tissue Bank was completed for each of the five species. Further discussions with staff from the Oceanic Fisheries Program (OFP) of the Pacific Community assisted with identifying the spatial distribution of samples held in the WCPFC Tuna Tissue Bank, their numbers and suitability for use in the project. Two applications for access to samples held in the WCPFC Tuna Tissue Bank were approved by the WCPFC Secretariat and staff from the Oceanic Fisheries Program (OFP) of the Pacific Community have facilitated access to the samples associated with those applications. Historical samples of bigeye, yellowfin, striped marlin and albacore held by CSIRO are being utilised by the project.

Collection of contemporary tissue samples

Collection of contemporary samples from the ETBF was initiated in late 2016 with samples from bigeye and yellowfin tuna, and swordfish collected from fishers operating in the ETBF. Samples from striped marlin from the New Zealand region are being collected by Blue Water Marine Research, with the first year of sampling completed.

Despite efforts, samples could not be obtained for swordfish from two sites external to the ETBF and within the WCPFC area. Collection of striped marlin was only possible from only two (ETBF and New Zealand) of three sites within the WCPFC. Collection from a third site is still pending.

The spatial sampling structure for the project and samples included in the project based on historical and contemporary collection of samples is provided in Table 1.

Genetic sequencing

DNA extraction and DNA profiling, using the DArTseq™ technique, has either been completed or is underway for all samples in hand (see also Table 1).

Quality control processes

Sample quality

Preliminary quality tests of DNA extractions from samples identified a number of samples from the WCPFC Tuna Tissue Bank that were not suitable for sequencing. This was because some tissue samples had degraded to the point that very little high molecular weight DNA could be extracted, which is necessary for the DArTseq™ technique, requiring replacement of poor quality individuals by others in the WCPFC Tuna Tissue Bank. Degradation of DNA in tissue samples can occur for a number of reasons including from poor care of fish from which samples are collected (e.g. market fish left exposed to the sun), poor handling of samples on vessels (e.g. samples left out on the deck) or during transit from vessels to archives (e.g. thawing of samples during transit) and repeated freeze thaw cycles that may occur as a result of multiple subsampling of tissues). Metadata associated with those tissues in which DNA degradation had occurred suggest a mixture of these factors likely contributed to the poor quality of samples archived.

Quality control of sequencing data

DNA profiles will be examined for consistency of genotyping parameters including: i) comparison of call rate of individuals versus average per locus; ii) total sequencing reads; iii) number of loci with read depth >7 counts (reference and SNP alleles combined) and; iv) departure from Hardy-Weinberg equilibrium across all individuals at a locus. Individuals and loci not matching average or expected values are deemed to be low quality data and will be discarded from the data set.

Table 1. Spatial structure and status of project samples.

Species	Location	Years	Status
Albacore tuna	ETBF	2	collected and sequenced
	New Caledonia	2	collected and sequenced
	New Zealand	2	collected sequencing underway
Bigeye tuna	ETBF	2	collected, sequencing underway
	Marshall Islands	2	collected and sequenced
	Solomon Islands	2	collected and sequenced
Striped marlin	ETBF	2	collected, sequencing underway
	New Zealand	2	collection underway
Swordfish	ETBF	2	collected and sequenced
Yellowfin tuna	ETBF	2	collected and sequenced
	Fiji	2	collected, sequencing underway
	Marshall Islands	2	collected, sequencing underway

Next steps

Finalisation of the collection of samples from striped marlin is ongoing both in the ETBF and in New Zealand with one further year of samples to be collected. As samples are collated, they will be submitted for DNA sequencing.

Quality control routines and initial runs of specialised models developed by CSIRO for stock structure discrimination and assignment will commence in the second half of 2018.

Intended outcomes

The information provided by this project will enable improved stock structure considerations used in domestic and regional pelagic fisheries scientific advice and management. Conducting stock assessments and implementing management on spatial units that reflect the underlying biology of the population structure should reduce the risk of over-fishing smaller and less productive stocks, while potentially enabling higher exploitation of larger and more productive stocks. In the Australian domestic context, this will allow for the updating of the harvest strategy currently used in the management of the ETBF with operating models that have increased accuracy and precision.

Reporting

Updates on the project in the form of information papers to the WCPFC scientific committee have been provided in 2016, 2017 and 2018 and a report on use of the samples from the Tissue Bank has been provided to the WCPFC Secretariat in 2018. We anticipate providing the WCPFC scientific committee with results from the project in 2019. A final report will be produced for submission to the Australian Government Fisheries Research Corporation in mid-2019 and a number of associated peer review publications produced, which will be forwarded on to the WCPFC.

Suggestions for future planning in association with the WCPFC Tuna Tissue Bank

Large scale stock structure investigations based on sequencing technologies require three key requirements of samples to be met in order to ensure rigour to results:

(i) Adequate sample sizes
(ii) Establishment of temporal stability in results

(iii) Verification of the provenance of samples

Power analysis carried out by CSIRO (unpublished) suggests that in order to maximise assignment rates for stock structure discrimination, sample collections should aim for a minimum of 50 fish from each location. Furthermore, each sample collection should be obtained from two time points separated by a minimum of 12 months to ensure that any observed spatial differentiation is not a result of a random sampling artefact. Sampling across multiple years also establishes whether any observed spatial differentiation is temporally stable. Finally, the provenance of samples identified from a particular location should be ensured in order to avoid introducing "false" or additional assignments to locations not being considered by the study. This requires, particularly in the case of sampling from fish markets, a knowledge of where fishers providing fish to the market have been fishing and any tracking of transhipment processes.

The WCPFC Tuna Tissue Bank relies on samples collected under country observer programs, each with varying priorities associated with data and sample collection, aligned with each country's fisheries management processes, plans and capacities. Establishment of the WCPFC Tuna Tissue Bank and the regular collection of samples contained and being contributed by country members of the WCPFC is a major achievement of the WCPFC - without the efforts placed into the archive to date, projects such as this would not be achievable. Spatial analysis of the tissue samples in the Tuna Tissue Bank has however identified a number of areas that could potentially be focused on to better optimise the utility of the archive for future investigations of species stock structure across the WCPFC:

I. Species coverage

Tissues currently contained in the Tuna Tissue Bank largely reflect the composition and quantities of species caught across the WCPFC area. There is however a distinct lack of samples currently archived from billfish species and some of the other species assessed under the WCPFC (e.g. sharks). Greater focus on these species and an associated increase in samples from these species would allow for the utilisation of samples in establishing currently uncertain life history parameters (e.g. age and growth) as well as building sample collections for use in investigations of stock structure.

II. Spatial coverage

Tissues currently contained in the Tuna Tissue Bank to some extent reflect the distribution of the highest catches across the WCPFC Area. There are however particular regions where samples are virtually or completely non-existent. Greater focus on current spatial gaps in sample collection (including capacity development) would allow for more comprehensive spatial coverage of tissues archived, thereby facilitating spatial analyses of biological parameters as well as building sample collections for use in investigations of stock structure.

III. Sample sizes

Tissues currently contained in the Tuna Tissue Bank, whilst impressive overall, rapidly decline in numbers once distributed on the basis of species, sample type, spatial and temporal qualifiers. In particular, the utility of the Tuna Tissue Bank declines for stock structure investigations, such as those being carried out by this project, where there is an aim to identify adequate samples from a defined region within a year across a number of years. Greater focus on building tissue samples from a small number of regions across multiple years (these regions could vary through time) would facilitate temporal assessments of biological parameters across regions as well as building sample collections for use in investigations of stock structure.

Acknowledgements

The authors would like to thank the members of WCFPC for the significant efforts placed into developing the Tuna Tissue Bank and facilitation of this project. Pacific Community Oceanic Fisheries Programme staff, in particular Neville Smith and Francois Roupsard are thanked for their assistance with accessing samples and associated information from the WCPFC Tuna Tissue Bank. Gary Heilman (DeBrett Seafoods) and Pavo Walker (Walker Seafoods Australia) are thanked for their assistance in accessing samples from the ETBF, Julian Pepperell for advice and assistance in accessing samples from recreational fishers and John Holdsworth for assistance in accessing samples from New Zealand. This project is supported by funding from the Fisheries Research Development Corporation and CSIRO.

References

Aguilar, R.D., Perez, S.K.L., Catacutan, B.J.N., Lopez, G.V., Barut, N.C., Santos, M.D. (2015). Distinct yellowfin tuna (*Thunnus albacares*) stocks detected in Western and Central Pacific Ocean (WCPO) using DNA microsatellites. Working paper WCPFC-SC11-2015/SA-IP-11a prepared for the eleventh regular session of the Western and Central Pacific Fisheries Commission Scientific Committee, 5-13 August 2015, Pohnpei, Federated States of Micronesia.

Evans, K., Grewe, P., Kolody, D., Hillary, R., Foster, S., Timmiss, T., Lansdell, M., Hill, P. (2016). Connectivity of tuna and billfish species targeted by the Australian Eastern and Tuna Billfish Fishery with the broader western Pacific Ocean. Working paper SA-IP-15 prepared for the twelfth regular session of the Western and Central Pacific Fisheries Commission Scientific Committee, 3-11 August 2016, Bali, Indonesia.

Grewe, P.M., Feutry, P., Hill, P.L., Gunasekera, R.M., Schaefer, K.M., Itano, D.G., Fuller, D.W., Foster, S.D., Davies, C.R. (2015). Evidence of discrete yellowfin tuna (*Thunnus albacares*) populations demands rethink of management for this globally important resource. Scientific Reports 5: 16916. doi: 10.1038/srep16916.

Grewe, P.M., Irianto, H.E., Proctor, C. H., Adam, M.S., Jauhary, A.R., Schaefer, K., Itano, D., Evans, K., Killian, A. Davies, C.R. (2016). Population structure ad provenance of tropical tunas: recent results from high throughput genotyping and potential implications for monitoring and assessment. Working paper SA-IP-01 prepared for the twelfth regular session of the Western and Central Pacific Fisheries Commission Scientific Committee, 3-11 August 2016, Bali, Indonesia.