SCIENTIFIC COMMITTEE
SIXTEENTH REGULAR SESSION

ELECTRONIC MEETING
11-20 August 2020

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

WCPFC-SC16-2020/SA-IP-16

Karen Evans[1], Peter Grewe[1], Scott Foster[2], Thierry Gosselin[3], Rasanthi Gunasekera[1], Mark Fitchett[4], John Holdsworth[5], Chi Hin Lam[6], Matt Lansdell[1], Molly Lutcavage[6], Steve Meredith[7], Francois Roupsard[8], Toni Ruchimat[9], Saiasi Sarau[10], Clay Tam[6], Marino Wichman[10].

[1] CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart Tasmania 7001, Australia
[2] Data61, CSIRO, GPO Box 1538, Hobart Tasmania 7001, Australia
[3] Department of Biology, L*'Université Laval, Ville de Quebec,* 2325 Rue de l'Université, Québec, QC G1V 0A6, Canada
[4] Western Pacific Regional Fishery Management Council**,** 1164 Bishop Street, Suite 1400, Honolulu, Hawai'i 96813 USA
[5] Blue Water Marine Research, PO Box 402081, Tutukaka, New Zealand
[6] Pacific Islands Fisheries Group, 150 Hamakua Drive PBN# 430, Kailua, Hawai'i 96734 USA
[7] Bay Packers, 5-11 Tyne St, Mt Maunganui, New Zealand
[8] The Pacific Community, B.P. D5, 98848 Noumea, New Caledonia
[9] Research Institute for Marine Fisheries, Cibinong, Indonesia
[10] Ministry of Marine Resources, PO Box 85, Avarua, Rarotonga, Cook Islands

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

Karen Evans[1], Peter Grewe[1], Scott Foster[2], Thierry Gosselin[3], Rasanthi Gunasekera[1], Mark Fitchett[4], John Holdsworth[5], Chi Hin Lam[6], Matt Lansdell[1], Molly Lutcavage[6], Steve Meredith[7], Francois Roupsard[8], Toni Ruchimat[9], Saiasi Sarau[10], Clay Tam[6], Marino Wichman[10].

[1]CSIRO Oceans and Atmosphere, GPO Box 1538, Hobart Tasmania 7001, Australia
[2]Data61, CSIRO, GPO Box 1538, Hobart Tasmania 7001, Australia
[3]Department of Biology, L'*Université Laval, Ville de Quebec,* 2325 Rue de l'Université, Québec, QC G1V 0A6, Canada
[4]Western Pacific Regional Fishery Management Council**,** 1164 Bishop Street, Suite 1400, Honolulu, Hawai'i 96813 USA
[5]Blue Water Marine Research, PO Box 402081, Tutukaka, New Zealand
[6]Pacific Islands Fisheries Group, 150 Hamakua Drive PBN# 430, Kailua, Hawai'i 96734 USA
[7]Bay Packers, 5-11 Tyne St, Mt Maunganui, New Zealand
[8]The Pacific Community, B.P. D5, 98848 Noumea, New Caledonia
[9]Research Institute for Marine Fisheries, Cibinong, Indonesia
[10]Ministry of Marine Resources, PO Box 85, Avarua, Rarotonga, Cook Islands

Abstract

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. The distribution ranges of these species are known to extend well beyond Australian waters and fish caught in the ETBF are considered to form part of a wider Western and Central Pacific Ocean (WCPO) population. Accordingly, regional stock assessments carried out for the Western and Central Pacific Fisheries Commission (WCPFC) assume that populations are single interconnected stocks. However, the specifics on connectivity between species caught in the ETBF with the WCPO is still a major source of uncertainty and biological, tagging and fisheries data suggest that there may be some structure to stocks within the region. Using high throughput genetic sequencing of total genomic DNA derived from yellowfin, bigeye and albacore tuna, swordfish and striped marlin collected from three sites (the ETBF and two outside the ETBF in the WCPO), co-dominant single nucleotide polymorphism (SNP) markers were identified. Purpose built quality control pipelines allowed for the discrimination of poor quality and compromised (e.g. through DNA cross contamination) samples. Mixture models were then used to investigate the presence of one or more genetic populations for each species. This paper will outline the results from the analyses of the five species, providing key insights into their contemporary connectivity within the context of current approaches to the assessment of stocks under the WCPFC. It also provides guidance on future planning for similar studies that might investigate population connectivity more broadly across the region, activities that have been recommended by the WCPFC as a priority for informing for future stock assessments.

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 project funded through the Fisheries Research Development Corporation on behalf of the Australian Government and the CSIRO (see Evans et al. 2016; 2017; 2018; 2019) 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; Grewe et al. 2019; Proctor et al. 2019; Davies et al. 2020). 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 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 (e.g. striped marlin, swordfish), 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. 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 (see Table 1, Figure 1).

DNA extraction

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. Gel runs were visually inspected as a first-pass qualitative check of the quality of the DNA in each sample. Samples that were qualitatively assessed as containing minimal amounts of DNA or highly denatured DNA were removed and did not progress to sequencing.

Genetic sequencing

DNA aliquots were shipped to Diversity Array Technologies in Canberra where DNA complexity reduction and library construction was performed prior to sequencing.

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 (data not presented). 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 single nucleotide polymorphism (SNP) and SilicoDArT (presence/absence of restriction fragments in representation) calling algorithms (DArTsoft14). For the purposes of the study in which the WCPFC samples were contributing to (see Evans et al. 2018), only co-dominant SNP-DArT markers were used for population analysis.

Species identification

Identification of swordfish, and striped marlin were confirmed using mitochondrial tests described by Innes et al. (1998). Identification of albacore, bigeye, and yellowfin tuna species were confirmed following restriction digestion of a mitochondrial PCR amplicon (PCR-RFLP) as described by Chow and Inoue (1993) with further modifications described by Takeyama et al. (2001). Size specific banding patterns representing restriction-fragment-length-polymorphisms (RFLPs) for all five species were resolved on 1.2% agarose gels using standard lab practices.

Quality control

A step wise process for data quality control using the package radiator (Gosselin 2017) was carried out at the individual markers and sample levels. Marker filtering includes marker reproducibility, identification of monomorphic markers, identification of common markers (these are markers that are present among all individuals), minor allele counts (which eliminates sequencing artefacts), minimum and maximum read depth (which is a reliability index of DNA quality and also identifies repetitive DNA which are not single copy genes – for example junk DNA in the genome), the proportion individuals that don't have a genotype at a locus, the quality of the sequencing run, the number of SNPs at a locus (addresses whether there are SNPs from different parts of the chromosome that have similar sequences) and whether loci comply with assumption of Hardy Weinberg equilibrium (Andrews 2010). Individual samples were filtered at three key steps: 1. missing data; 2. average heterozygosity; 3.removal of highly similar/duplicate genotypes.

Population modelling

Population modelling using a mixture model was based on the method outlined in Foster et al. (2018) and implemented in the R package stockR (Foster 2018). The model assumes that each sample belongs to one of K genetic groups ($K \geq 1$ and is an integer), and for this study the purpose of the analysis is twofold: 1) To assign a probability of assignment of each sample to each of K putative genetic groups and 2) to provide information about how many genetic groups there are likely to be. For a given K, the modelling approach uses maximum likelihood to estimate the allele frequencies within each population, and then uses model summaries (posterior membership probabilities) to estimate the chance of each sample belonging to each genetic group (Foster et al. 2018).

Two approaches are utilised in order to determine an appropriate K for the data of each species:

1. Information Criteria: Two information criteria (AIC and BIC) are calculated from the fitted model with the number of groups (K) that minimised the information criterion identified as providing the best fit.
2. Cross Validation (stability): 5-fold cross validation is used to evaluate how quickly the predictive performance of the model diminishes as more stocks were added. To obtain the cross-validation

statistics B = 1000 holdout samples are used. Formally, this is not a cross-validation procedure, but it is closely related. It differs from cross validation as the target of prediction, the group assignment probability, is not observed within the data themselves. In their place, the predicted assignment probabilities from the model fitted to all the data combined is used as the prediction target. So, in this sense this analysis is looking at the *stability* of the assignment probabilities due to subsampling and permutation.

Uncertainty in the assignment probabilities was quantified in the models by using the Bayesian bootstrap methods described in Foster et al. (2018). The uncertainty is graphically portrayed, along with the results, using bar plots. Individual bars represent the probability of assignment of a fish to each genetic grouping (K) plotted as a stacked bar with different colours for each group. The amount of colour saturation of the plotted colour bar is taken from the amount of uncertainty in the estimated probability for that sample in that group. If an estimated probability is highly uncertain, then the bar is (nearly) white, whereas if it is quite certain then the bar is plotted with a solid colour. The amount of uncertainty is quantified by the width of the 95% confidence interval – with an interval of 1 being the highest possible for a probability estimate.

It is important to note that the sampling regions are not used in this analysis, only in presentation. The only information included in the modelling process are the genetic data themselves. This means that the analysis does not intentionally seek spatially consistent groupings, but if there is a real spatial signal then this should show in any case.

Results

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 and Figure 1.

Accessing samples from broadbill swordfish from two sites within the WCPFC area was particularly problematic and although a New Zealand processor was identified by late 2018, a poor fishing season resulted in no samples being collected from New Zealand in 2019. This poor fishing season also impacted sampling from striped marlin from New Zealand, resulting in only 15 samples able to be collected. Lockdowns associated with the COVID-19 pandemic in New Zealand and a shutting down of all broadbill swordfish fishing operations in the Cook Islands further impacted sampling. As a result, only those samples collected across one year from New Zealand and the ETBF are included here. Sampling will continue from New Zealand in 2021 and once operations resume in the Cook Islands sampling will continue across 2020 and 2021, with analysis of these samples planned in 2021.

Given the nature of the collections from which samples were derived, samples comprised a mix of sexes, lengths and therefore age classes/cohorts and potentially reproductive state. Those lengths that were collected were: albacore: 48 – 106 cm, bigeye: 35 – 148 cm, broadbill swordfish: 110 – 160 cm; yellowfin: 52 – 158 cm; striped marlin: 121 – 241 cm. Note not all fish were measured for their length, length of tunas were length to caudal fork, lengths of broadbill swordfish were trunk lengths (head, internal organs and tail removed) and lengths of striped marlin were orbital to fork length.

Quality control processes

The number of samples removed at each of the quality control steps (quality of DNA, species identification, missing data, genome-wide average heterozygosity, highly similar/duplicate genotypes) are detailed in Table 1.

Population modelling

Albacore tuna

The outputs from the mixture model and cross validation identified the number of genetic groups occurring across samples as most supportive of a K=1 (Figure 2). As such, the results suggest very little genetic differentiation among the three sampling locations (ETBF, New Caledonia and New Zealand), which is consistent with our ability to resolve a single genetic grouping of albacore tuna with regard to the three sites among the years examined.

Bigeye tuna

The outputs from the mixture model and cross validation identified the number of genetic groups occurring across samples as most supportive of a K=1 (Figure 3). Probability of assignment was consistent across years at all three sites (ETBF, Marshall Islands, Solomon Islands). Given the information criteria and cross validation results, the data so not support discrimination of more than one genetic group of bigeye tuna across the three sites among the years examined.

Broadbill swordfish

The outputs from the mixture model and cross validation identified the number of genetic groups occurring across samples as most supportive of a K=1. (Figure 4). Probability of assignment was consistent across years in samples from the ETBF. Given the information criteria and cross validation results, the data so not support discrimination of more than one genetic group of broadbill swordfish across the three sites (ETBF Norfolk Island, New Zealand) among the years examined.

Striped marlin

The outputs from the mixture model and cross validation indicate that there may be K=2 genetic groups of striped marlin within these data, with the ETBF, NZ and Hawai'i sharing the first group (Figure 5). The second (less sampled group) was identified only from samples collected from Hawai'i. Probability of assignment was consistent across years in samples from the ETBF and NZ. Sampling of only one year from Hawai'i precluded any assessment of temporal stability in assignment to genetic groups from this site.

Yellowfin tuna

The outputs from the mixture model and cross validation identified the number of genetic groups occurring across samples as most supportive of a K=1 (Figure 6). Probability of assignment was consistent across years at all locations (ETBF, Fiji, Marshall Islands). Given the results from the mixture model and cross validation, the data so not support discrimination of more than one genetic group of yellowfin tuna across the three sites among the years examined.

Discussion

Connectivity of tuna and billfish species

The analyses presented here build on previous tagging and genetic studies to provide further insights into the connectivity of the ETBF with adjacent waters and areas within the greater western and central Pacific Ocean (Domeier 2006; Evans et al. 2008; Evans et al. 2011; Bradman et al. 2011; Evans et al. 2014; Grewe et al. 2015; Tracey and Pepperell 2018; Mamoozadeh et al. 2020).

From the perspective of the ETBF, the results suggest a substantial level of connectivity and mixing of all five species between each of the areas investigated. The data exhibited little discernible genetic differentiation between areas for albacore, bigeye and yellowfin tunas and broadbill swordfish. The results from striped marlin indicate that there may be two genetic groups, with the ETBF, NZ and Hawai'i sharing the first group. The second (less sampled group) was identified only

from samples collected from Hawai'i. The consistent absence in the ETBF and New Zealand of the second genetic group found in Hawai'i indicates a proportion of fish from Hawai'i do not contribute to the ETBF fishery and potentially represent a northern hemisphere population that doesn't migrate south of the equator. The stability of results across years for most species and locations indicate that the identified genetic groupings are temporally stable. Where multiple years of samples were unavailable, similarities between the results presented here with those reporting by other authors, suggest that groupings identified are likely to be temporally stable.

It should be noted that these results *only apply to the sites* included for each of the species in this study and therefore *cannot* be extrapolated across the wider western and central Pacific Ocean region. Although a number of other genetic population studies including samples from the western and central Pacific Ocean and utilising SNPs have been conducted (e.g. Grewe et al. 2015; Laconcha et al. 2015; Anderson et al. 2019a; Proctor et al. 2019; Mamoozadeh et al. 2020), differences in sampling, sequencing methods, quality control pipelines and modelling approaches mean that it is inappropriate to group results. Further consistent sampling and analysis of sites across the western and central Pacific would be needed to investigate whether the results presented here extend to other locations across the western and central Pacific region or whether greater genetic differentiation is discernibly present across the region (see also below section on sampling design). Further sampling of broadbill swordfish from New Zealand and the Cook Islands across 2020 and 2021 and planned for analysis in 2021 will provide further insights into the connectivity of this species across the western and central Pacific Ocean.

The genetic groupings identified here, however are consistent with previous genetic investigations into the population structure of albacore, bigeye and yellowfin tunas. Albacore tuna sampled from New Caledonia and French Polynesia have been observed to demonstrate no significant heterogeneity on the basis of both microsatellite DNA markers and SNPs derived from the same samples (Montes et al. 2012, Albaina et al. 2013, Laconcha et al. 2015). No significant differentiation of bigeye tuna mitochondrial DNA has been observed from samples collected across the equatorial region from 170˚W to 150˚E (Wu et al. 2014). Similarly, observations based on mitochondrial DNA and microsatellite loci were unable to demonstrate Pacific-wide population heterogeneity of bigeye tuna (Grewe and Hampton 1998) and recent analyses of SNPs from both bigeye and yellowfin tunas report that sample sites in close proximity to each other in both the Pacific and Indian Oceans appear to be more genetically similar than those separated at ocean basin scale distances (Grewe et al. 2015; Proctor et al. 2019; Davies et al. 2020).

Genetic differentiation that has been reported on albacore, bigeye and yellowfin tunas has been observed across larger spatial scales than the spatial scales included in this study. Adaptive SNP loci from albacore tuna caught across the French Polynesia exclusive economic zone have been reported as being differentiated from those from New Caledonia, New Zealand and Tonga (Anderson et al. 2019a). However, the sampling design of this study (low sample sizes, large spatial dispersion of samples, and utilisation of samples from a single year) limits the ability to determine the stability or representativeness of these results. Meta-analysis of studies investigating the population structure of yellowfin tuna found that the larger geographic region encompassed in studies, the increased potential for finding genetic differentiation (Anderson et al. 2019b). This is largely in line with commonly proposed models for albacore, bigeye and yellowfin tunas of isolation by distance (e.g. Laconcha et al 2015). Such models assume a continuous population facilitated through the exchange of genes among individuals in close proximity to one another (Moore et al. 2020a).

An investigation into the origins of yellowfin tuna caught off the east coast of Australia using otolith microchemistry reported linkages between yellowfin tuna across the western Pacific Ocean with

associations between fish caught in the ETBF with fish from Indonesia and the Solomon Islands (Gunn et al. 2002). The results presented here suggest that there may be broader associations between yellowfin tuna caught in the ETBF with those in the western Pacific Ocean with little discernable differentiation observed with fish from Fiji and the Marshall Islands.

While investigations of the population structure of broadbill swordfish globally have identified molecular variation between ocean basins (Alvarado Bremer 1996; Rosel and Block 1996; Kotoulas et al. 2007), only a small number of studies have investigated structuring within ocean basins to date. Of those that have, some indication of structuring has been observed in the Pacific Oceans at the basin-scale, with low levels of mitochondrial gene flow. This gene flow appears to have a ⊃-shaped pattern, with connectivity of animals east-west in the Northern and Southern Hemispheres and connections across the equatorial zone along the west coast of the Americas (Reeb et al. 2000). This is consistent with larval distributions (Grall et al. 1983; Nishikawa et al. 1985) and the hypothesis of separate stocks in the north and southwest Pacific Ocean (Sakagawa and Bell 1980). DNA sequence polymorphisms from swordfish collected across the Pacific Ocean identified fish from the south-east Pacific Ocean as genetically different to all other locations sampled (Alvarado-Bremer et al. 2006). In addition, fish from the north-east Pacific Ocean were observed to be different to those collected from around Hawai'i, which were in turn differentiated from those in the south-west Pacific Ocean (Alvarado-Bremer et al. 2006). Mitochondrial differentiation has been reported from samples collected from waters to the east of Australia and north of New Zealand, although this differentiation was not significant after post-hoc correction (Kasapidis et al. 2008). Our results suggest that at least within the Coral Sea/Tasman Sea region, that there is little genetic differentiation between fish caught across the region. Further sampling across the WCPFC area would be needed to determine whether there is structuring occurring at spatial scales smaller than those observed in Reeb et al. (2000) and Alvarado Bremer et al. (2006) and larger than that observed in this study. Although samples from the Cook Islands were not able to be included in the analyses presented here, we anticipate further collection of samples across the second half of 2021 and first half of 2021. Samples collected will be included in analyses planned during 2021 and may provide further insights into the presence of any genetic structuring across the western and central Pacific Ocean.

Previous studies investigating the population structure of striped marlin using microsatellite loci have identified genetic diversity between samples collected from the ETBF and those collected from Hawai'i (McDowell and Graves 2008). However, more recent investigation of SNPs from striped marlin identified that samples collected from the ETBF and New Zealand clustered together and that there appeared to be two genetic groupings amongst samples collected from Hawai'i, one of which was similar to the ETBF/New Zealand grouping (Mamoozadeh et al. 2020). The presence of two genetic populations in the waters of Hawai'i has been proposed previously (Bromhead et al. 2004; Purcell and Edmands 2011) and the results presented here lend further support to this hypothesis. The samples included in this study were collected from the Honolulu fish market and lack information on the locations of capture. This precludes any investigation into whether the two genetic groupings are derived from different fishing regions or if individuals assigned to the two groups are dispersed throughout broadly spaced fishing locations. Nevertheless, the results presented here confirm the observation made by Mamoozadeh et al. (2020) and suggest some spatial mixing of a component of the striped marlin caught in waters around Hawai'i with those caught in the western Pacific Ocean.

Considerations on sample quality and sampling design

Degradation of DNA in tissue samples can occur for a number of reasons including from poor care of fish prior to sampling (e.g. market fish left exposed to the sun), poor handling of samples on vessels (e.g. samples left out on the deck) or degradation during transit from vessels or the market to archives (e.g. thawing of samples during transit), repeated freeze- thaw cycles that may occur as a result of multiple subsampling of tissues or poor storage of tissues. Without clear information on how individual samples were handled, it is difficult to determine what may have caused the degradation of those tissues from striped marlin and yellowfin tuna observed among samples examined in the current study. Clear standard operating protocols associated with sampling, handling and archival of tissues and a commitment to uphold those protocols would assist in avoiding this issue and ensure that efforts placed into the collection and archival of samples are maximised in terms of the future utility of samples held in collections.

High heterozygosity observed across a number of samples from various locations is reflective of sample cross contamination. Cross contamination of samples can occur at the point of sampling, during handling or during subsampling and often is the symptom of poor tissue sampling skills or inadequate cleaning protocols (e.g. not cleaning the knife or scalpel blade in between samples, not cleaning hands when handling multiple samples). This also highlights the need for clear standard operating procedures associated with sampling and sub-sampling and application of these across all handling processes.

In all areas of science, it is much easier to show things are different when there is a large difference. In this study, we attempted to differentiate differences that are, in hindsight, small. The sample sizes included (see Table 1) in this study are not excessive and in some cases we did not achieve our original intended sample numbers. However, the sample numbers achieved are in line with, and in many cases exceed (per location, per year and also overall), other studies on tunas and billfish that have shown believable differences (Grewe et al. 2015; Proctor et al. 2019; Davies et al. 2020). Including larger sample sizes here would provide more evidence, but these sample sizes are past the point of providing diminishing returns – the effect of increasing sample sizes past those in this study do not change error rates substantially (Foster et al. unpublished data).

When this project was originally conceived, a key component of the sampling design for the project was to include samples from adult individuals in spawning condition. This was only possible for one group of samples from the ETBF, (2014 yellowfin tuna) as determining reproductive stage of fish sampled from processors was not possible due because fish had been gilled and gutted at sea. Samples from the WCPFC Tropical Tuna Tissue Bank were not extensive enough to permit exclusive sampling of adults. Similarly, sample numbers of striped marlin collected from Hawai'i were not extensive enough to only include adults in this analysis.

The mixed nature of the samples therefore constrained the questions that could be proposed and investigated by this project, namely "does the genetic signature of fish sampled from the three sites vary to the extent that they can be identified as different". This should not be confused with questions that might be related to the investigation of distinct spawning populations and evolutionary gene flow. Rather, the samples and methods applied here provide some insights into contemporary mixing of individuals on the fishing grounds from which samples were derived.

In order to establish spatially explicit understanding of stock structure in a species, any methods employed must be able to establish two key measures: (i) provenance (where an animal is sourced from); and (ii) the degree of mixing of the population the animal is sourced from with other populations. Both require unique sampling regimes that need to include a spatial as well as a

temporal component to ensure that any identified genetic structure is robust and reflects that present in the population.

To be able to determine if multiple spawning populations for individual species exist, sampling would need to be structured in such a way that actively spawning fish (or those that are running ripe) from distinct locations are sampled at the same time and across at least two time points or a period confirming the temporal stability of the observed degree of population structure. Spatial sampling also needs to occur on a large enough scale that realistically reflects potential populations, particularly where a spawning population might be dispersed rather than being discrete to particular site(s) within a region. This is not currently possible with the sample collections available and would require structured and dedicated sampling to achieve (see Moore et al. 2020a).

As part of annual reporting to the WCPFC Scientific Committee, a number of recommendations have been identified that might be useful in guiding how the WCPFC Tropical Tuna Tissue Bank could be modified to better support population structure studies. These were provided in SA-IP-13 presented to the twelfth regular session of the Scientific Committee, 2019.

Conclusions

The results presented here build on substantial investments into understanding the connectivity of albacore tuna, bigeye tuna, broadbill swordfish, striped marlin and yellowfin tuna across the western Pacific Ocean. They have direct relevance to the current revisions of harvest strategies for broadbill swordfish and striped marlin in the ETBF, informing operating models being developed and used, particularly in terms of mixing scenarios. Should any potential development of harvest strategies for albacore, bigeye and yellowfin tunas be considered, similarly these results presented here provide relevant information for considering mixing rates of fish in the ETBF with the western Pacific Ocean region.

More broadly, the results of this project have generated substantial discussion focused around the future development of sampling programs associated with the WCPFC Tropical Tuna Tissue Bank and supported by the WCPFC observer program. Initial discussions focused on sampling designs for supporting population structure studies were held between CSIRO, AFMA, ABARES and the Pacific Community in February 2020 and are ongoing.

Importantly, the results presented here and their relevance to other studies investigating the population structure of the five species highlight that care needs to be taken in extrapolating results from a limited number of locations to the wider Pacific Ocean. Variability in results between studies suggest that in order to determine a comprehensive understanding of the population structure of species of relevance, substantive spatial sampling across the western and central Pacific and some temporal duplication of sampling is required. This will require dedicated efforts placed towards the development, design and carrying out of such a program. Initial work in understanding what would be required for establishing the population structure of tuna species in the western and central Pacific Ocean was the focus of a workshop held at the Pacific Community in 2018 to which this project's staff contributed to (published in Moore et al. 2020a, 2020b).

Acknowledgements

We would also like to thank the members of Western and Central Pacific Fisheries Commission (WCPFC) for the significant efforts placed into developing the WCPFC Tropical Tuna Tissue Bank and

facilitation of this project. Pacific Community Oceanic Fisheries Programme staff, in particular Neville Smith and Caroline Sanchez are thanked for their assistance with accessing samples and associated information from the WCPFC Tropical Tuna Tissue Bank. Gary Heilman (De Brett Seafoods) and Pavo and Heidi Walker (Walker Seafoods Australia) are thanked for their assistance in accessing samples from the Eastern Tuna and Billfish Fishery. We thank Anung A. Widodo (Centre of Fisheries Research, Indonesia) and Craig Proctor for facilitating access to samples collected from the Solomon Islands under the ACIAR project FIS/2009/059. The Tropical Tuna Resource Assessment Group are thanked for providing useful feedback and suggestions to the project throughout its lifetime. This project is supported by funding from the Fisheries Research Development Corporation and CSIRO.

References

Aguilar RD, Perez SKL, Catacutan BJN, Lopez GV, Barut NC, Santos MD. 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.

Albaina A, Iriondo M, Velado I, Laconcha U, Zarraonaindia I, Arrizabalaga H, Pardo MA, Lutcavage M, Grant WS, Estonba A. 2013. Single nucleotide polymorphism discovery in albacore and Atlantic bluefin tuna provides insights into worldwide population structure. Animal Genetics 44: 678 – 692. doi:10.1111/age.12051.

Alvarado Bremer JR, Hinton MG, Greig TW. 2006. Evidence of spatial genetic heterogeneity in Pacific swordfish (*Xiphias gladius*) revealed by the analysis of *LDH-A* sequences. Bulletin of Marine Science 79: 493 – 503.

Alvarado Bremer JR, Mejuto J, Greig TW, Ely, B. 1996. Global population structure of swordfish (*Xiphias gladius* L.) as revealed by analysis of the mitochondrial DNA control region. Journal of Experimental Marine Biology and Ecology 197: 295 – 310.

Anderson G, Hampton J, Smith N, Rico C. 2019b. Indications of strong adaptive population genetic structures in albacore tuna (*Thunnus alalunga*) in the southwest and central Pacific Ocean. Ecology and Evolution 9: 10354 – 10364. doi:10.1002/ece3.5554.

Anderson G, Holland E, Rico C. 2019a. Meta-analysis of factors influencing population differentiation in yellowfin tuna (*Thunnus albacares*). Reviews in Fisheries Science and Aquaculture 27: 517 – 532. Doi: 10.1080/23308249.2019.1633270.

Andrews C. 2010. The Hardy-Weinberg principle. Nature Education Knowledge 3: 65.

Bradman H, Grewe P, Appleton B. 2011. Direct comparison of mitochondrial markers for the analysis of swordfish population structure. Fisheries Research 109: 95 – 99. doi:10.1016/j.fishres.2011.01.022.

Bromhead D, Pepperell J, Wise B, Findlay J. 2004. Striped marlin: biology and fisheries. Final report to the Australian Fisheries Management Authority. Bureau of Rural Sciences, Canberra.

Chow S, Inoue S. 1993. Intra- and interspecific restriction fragment length poly- morphism in mitochondrial genes of *Thunnus* tuna species. Bulletin of National Research Institute of Far Seas Fisheries 30: 207 – 225.

Davies CR, Marsac F, Murua H, Fraile I, Fahmi Z, Farley J, Grewe P, Proctor C, Clear N, Eveson P, Lansdell M, Aulich J, Feutry P, Cooper S, Foster S, Rodríguez-Ezpeleta N, ArtetxeArrate I, Krug Mendibil I, Agostino L, Labonne M, Nikolic N, Darnaude A, Arnaud-Haond S, Devloo-Delva F, Rougeux C, Parker D, Diaz-Arce N, Wudianto Ruchimat T, Satria F, Lestari P, Taufik M, Priatna A, Zamroni A. 2020. Study of population structure of IOTC species and sharks of interest in the Indian Ocean using genetics and microchemistry. Final Report to IOTC.

Domeier ML. 2006. An analysis of Pacific striped marlin (*Tetrapturus audax*) horizontal movement patterns using pop-up satellite archival tags. Bulletin of Marine Science 79: 811 – 825.

Evans K, Abascal F, Kolody D, Sippel T, Holdsworth J, Maru P. 2014. The horizontal and vertical dynamics of swordfish in the South Pacific Ocean. Journal of Experimental Marine Biology and Ecology 450: 55 – 67. doi:10.1016/j.jembe.2013.10.025.

Evans K, Grewe P, Foster S, Gosselin T, Gunasekera R, Lansdell M. 2019. Connectivity of tuna and billfish species targeted by the Australian Eastern Tuna and Billfish Fishery with the broader Western Pacific Ocean. Information paper SA-IP-13 presented to the fifteenth regular session of the Western and Central Pacific Fisheries Commission

Evans K, Grewe P, Gunasekera R, Lansdell M. 2017. Connectivity of tuna and billfish species targeted by the Australian Eastern Tuna and Billfish Fishery with the broader Western Pacific Ocean. Information paper SA-IP-03 presented to the thirteenth regular session of the Western and Central Pacific Fisheries Commission Scientific Committee, 9-17 August 2017, Rarotonga, Cook Islands.

Evans K, Grewe P, Gunasekera R, Lansdell M. (2018). Connectivity of tuna and billfish species targeted by the Australian Eastern Tuna and Billfish Fishery with the broader Western Pacific Ocean. Working paper SA-IP-04 presented to the fourteenth regular session of the Western and Central Pacific Fisheries Commission Scientific Committee, 8-16 August 2018, Busan, Republic of Korea.

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.

Evans K, Langley A, Clear NP, Williams P, Patterson T, Sibert J, Hampton J, Gunn JS. 2008. Behaviour and habitat preferences of bigeye tuna (*Thunnus obesus*) and their influence on longline fishery catches in the western Coral Sea. Canadian Journal of Fisheries and Aquatic Science 65: 2427 – 2443.

Evans K, Patterson T, Pedersen M. 2011. Movement patterns of yellowfin tuna in the Coral Sea region: defining connectivity with stocks in the western Pacific Ocean region. Final report to the Australian Fisheries Management Authority. CSIRO Marine and Atmospheric Research, Hobart.

Foster SD. 2018. stockR: Identifying Stocks in Genetic Data. R package version 1.0.68.

Foster SD, Feutry P, Grewe PM, Berry O, Hui FKC, Davies CR. 2018. Reliably discriminating stock structure with genetic markers: Mixture models with robust and fast computation. Molecular Ecology Resources 18: 1310 – 1325.

Gosselin T. 2017. radiator: RADseq Data Exploration, Manipulation and Visualization using R.

Grall C, De Silva DP, Houde ED. 1983. Distribution, relative abundance and seasonality of swordfish larvae. Transactions of the American Fisheries Society 112: 235–246.

Grewe PM, Feutry P, Hill PL, Gunasekera RM, Schaefer KM, Itano DG, Fuller DW, Foster SD, Davies CR. 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 PM, Irianto HE, Proctor CH, Adam MS, Jauhary AR, Schaefer K, Itano D, Evans K, Killian A, Davies CR. 2016. Population structure ad provenance of tropical tunas: recent results from high throughput genotyping and potential implications for monitoring and assessment. Information 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.

Grewe PM, Kreuger CC, Aquadro CF, Bermingham E, Kincaid HL, May B. 1993. Mitochondrial DNA variation among lake trout (*Salvelinus namaycush*) strains stocked into Lake Ontario. Canadian Journal of Fisheries and Aquatic Science 50: 2397 – 2403.

Grewe PM, Wudianto, Proctor CH, Adam MS, Jauhary AR, Schafer K, Itano D, Evans K, Killian A, Foster S, Gosselin T, Feutry P, Aulich J, Gunasekera, R, Lansdell M, Davies CR. 2019. Population structure and connectivity of tropical tuna species across the Indo Pacific Ocean region. Information paper SA-IP-15 presented to the fifteenth regular session of the Western and Central Pacific Fisheries Commission Scientific Committee, 12-20 August 2019, Pohnpei, Federated States of Micronesia.

Gunn JS, Clear NP, Ward RD, Appleyard SA, Grewe PM, Innes BH. 2002. The origin of recruits to the east coast yellowfin tuna fishery and the delineation of the structure of yellowfin tuna stocks in the western Pacific. Final report to the Fisheries Research and Development Corporation. CSIRO Marine Research, Hobart.

Innes HB, Grewe P, Ward R. 1998. PCR-based genetic identification of marlin and other billfish. Marine and Freshwater Research 49: 383–88. doi:10.1071/MF98007.

Kasapidis P, Magoulas A, García-Cortés B, Mejuto M. 2008. Stock structure of swordfish (*Xiphias gladius*) in the Pacific Ocean using microsatellite DNA markers. Working paper WCPFC-SC4-2008/BI-WP-4 presented to the Western and Central Pacific Fisheries Commission Scientific Committee fourth regular session, 11 – 22 August 2008, Port Moresby, Papua New Guinea.

Kotoulas G, Mejuto J, Antoniou A, Kasapidis P, Tserpes G, Piccinetti C, Peristeraki P, García-Cortés B, Oikonomaki K, de la Serna JM, Magoulas A. 2006. Global genetic structure of swordfish (*Xiphias gladius*) as revealed by microsatellite DNA markers. ICCAT Collective Volume of Scientific Papers 61: 79 – 88.

Laconcha U, Iriondo M, Arrizabalaga H, Manzano C, Markaide P, Montes I, Zarraonaindia I, Velado I, Bilbai E, Goñi N, Santiago J, Domingo A, Karakulak S, Oray I, Estonba A. 2015. New nuclear SNP markers unravel the genetic structure and effective population size of albacore tuna (*Thunnus alalunga*). PLoS ONE 10: e0128247. Doi: 10.1371/journal.pone.0128247

Mamoozadeh NR, Graves JE, McDowell JR. 2020. Genome-wide SNPs resolve spatiotemporal patterns of connectivity within striped marlin (*Kajikia audax*), a broadly distributed and highly migratory pelagic species. Evolutionary Applications 13: 677 – 698. doi: 10.1111/eva.12892.

Montes I, Iriondo M, Manzano C, Arrizabalaga H, Jiménez E, Pardo MÁ, Goñi N, Davies CA, Estonba A. 2012. Worldwide genetic structure of albacore *Thunnus alalunga* revealed by microsatellite DNA markers. Marine Ecology Progress Series 471: 183 – 191. doi:10.3354/meps09991.

Moore BR, Adams T, Allain V, Bell J, Bigler M, Bromhead D, Clark S, Davies C, Evans K, Faasali U, Farley J, Fitchett M, Grewe PM, Hampton J, Hyde J, Leroy B, Lewis A, Lorrain A, MacDonald JI, Marie AD, Minte-Vera C, Natasha J, Nicol S, Obregon P, Pecoraro C, Bradley Phillip N, Scutt Phillips J, Pilling GM, Rico C, Sanchez C, Scott R, Stockwell B, Tremblay-Boyer L, Usu T, Williams AJ, Smith N. 2020a. Defining stock structure in four commercial tunas in the Pacific Ocean II: sampling considerations and future directions. Fisheries Research, doi:10.1016/j.fishres.2020.105524.

Moore BR, Bell J, Evans K, Farley J, Grewe PM, Hampton J, Marie AD, Minte-Vera, C, Nicol S, Scutt Phillips J, Pilling GM, Tremblay-Boyer L, Williams A, Smith N. 2020b. Defining stock structure in four commercial tunas in the Pacific Ocean I: current knowledge and key uncertainties. Fisheries Research, doi:10.1016/j.fishres.2020.105525.

Nishikawa Y, Honma M, Ueyanagi S, Kikawa S. 1985. Average distribution of larvae of oceanic species of scombrid fishes, 1956 – 1981. Far Seas Fisheries Research Laboratories S Series 12: 1 – 99.

Proctor CH, Lester RJG, Clear NP, Grewe PM, Moore BR, Eveson JP, Lestari P, Wujdi A, Taufik M, Wudianto, Lansdell MJ, Hill PL, Dietz C, Thompson JM, Cutmore SC, Foster SD, Gosselin T, Davies CR. 2019. Population structure of yellowfin tuna (*Thunnus albacares*) and bigeye tuna (*T. obesus*) in the Indonesian region. Final Report as output of ACIAR Project FIS/2009/059. Australian Centre for International Agricultural Research, Canberra.

Purcell CM, Edmands S. 2011. Resolving the genetic structure of striped marlin *Kajikia audax*, in the Pacific Ocean through spatial and temporal sampling of adult and immature fish. Canadian Journal of Fisheries and Aquatic Science 68: 1861 – 1875. doi:10.1139/F2011-104.

Reeb CA, Arcangeli L, Block BA. 2000. Structure and migration corridors in Pacific populations of the swordfish *Xiphias gladius*, as inferred through analyses of mitochondrial DNA. Marine Biology 136: 123 – 131.

Rosel PE, Block BA. 1996. Mitochondrial control region variability and global population structure in the swordfish, *Xiphias gladius*. Marine Biology 125: 11 – 22.

Sakagawa GT, Bell RR. 1980. Swordfish, *Xiphias gladius*. Pages 43 – 55 in Shomura RS (ed.). Summary report of the billfish stock assessment: Pacific resources. NOAA technical Memorandum NMFS-SWC5. US Department of Commerce, Seattle.

Takeyama, H., Chow, S., Tsuzuki, H., Matsunaga, T. (2001). Mitochondrial DNA sequence variation within and between Thunnus tuna species and its application to species identification. Journal of Fish Biology 58: 1646‑1657. doi: 10.1006/ jfbi.2001.1575.

Tracey S, Pepperell J. 2018. Understanding the movement, behaviour and post-capture survival of recreationally caught swordfish from southeast Australia – a pilot study. FRDC Project No 2015/022. University of Tasmania, Hobart.

Wu Z, Xu Q, Zhu J, Dai X, Xu L. 2014. Genetic population structure of the bigeye tuna Thunnus obesus in the central Pacific Ocean based on mtDNA Cyt*b* sequences. Fisheries Science 80: 415 – 426. Doi: 10.1007/s12562-014-0712-3.

Table 1. Samples by species and region with details of samples removed through the species identification and quality control processes.

Species	Region/EEZ	Year	Number samples received	DNA quality (qualitative)	Number genotypes (incl. replicates)	Incorrect species ID	Missing data	Heterozygosity	Similar genotypes	Final number
Albacore	Australia	2009	50	—	73	—	19	1	18 (15 technical replicates)	35
		2010	50	—	50	—	7	—	3	40
Albacore	New Caledonia	2013	25	—	36	—	—	1	11 (8 technical replicates)	24
		2014	37	—	44	—	—	—	11 (7 technical replicates)	33
		2016	45	—	64	—	—	30	18 (10 technical replicates)	16
Albacore	New Zealand	2008	47	—	63	—	6	5	20 (12 technical replicates)	32
		2010	47	—	47	—	—	5	9	33
Bigeye	Australia	2017	50	—	66	—	—	22	20 (2 technical replicates)	28
		2018	50	—	73	—	—	53	6 (all technical replicates)	14
Bigeye	Marshall Islands	2014	50	—	50	3	—	6	1	40
		2015	50	—	50	1	—	3	—	46
Bigeye	Solomon Islands	2013	56	—	56	—	—	2	7 (all technical replicates)	47

Species	Region/EEZ	Year	Number samples received	DNA quality (qualitative)	Number genotypes (incl. replicates)	Incorrect species ID	Missing data	Heterozygosity	Similar genotypes	Final number
		2014	49	—	112	—	—	1	70 (59 technical replicates)	51
Broadbill swordfish	Australia	2016 (Norfolk Island)	13	—	13	—	—	—	—	13
		2017 (ETBF)	48	—	51	1	—	1	3 (all technical replicates)	46
		2018 (ETBF)	31	—	31	—	—	—	—	31
Broadbill swordfish	Cook Islands	2019	11(collected)							
		2020								
Broadbill swordfish	New Zealand	2020	50		74	—	—	1	28 (23 technical replicates)	45
Striped marlin	Australia	1996	34	—	49	—	—	14	8 (all technical replicates)	27
		2017	41	—	51	—	—	2	9 (8 technical replicates)	40
Striped marlin	Hawai'i	2017	148	35	129	2	—	59	15	53
Striped marlin	New Zealand	2018	57	—	73	—	—	20	10 (all technical replicates)	43
		2019	15	—	15	—	—	1	—	14

Species	Region/EEZ	Year	Number samples received	DNA quality (qualitative)	Number genotypes (incl. replicates)	Incorrect species ID	Missing data	Heterozygosity	Similar genotypes	Final number
Yellowfin	Australia	2006	50	—	65	—	8	15	9 (all technical replicates)	33
		2013	85	—	118	—	—	—	34 (33 replicate samples or technical replicates)	84
Yellowfin	Fiji	2014	62	9	76	—	—	30	14 (12 technical replicates)	32
		2015	60	25	39		1	15	5 (all technical replicates).	18
Yellowfin	Marshall Islands	2014	63	13	51	1	—	3	1 (technical replicate)	46
		2015	52	2	58	3	—		9 (all technical replicates)	46

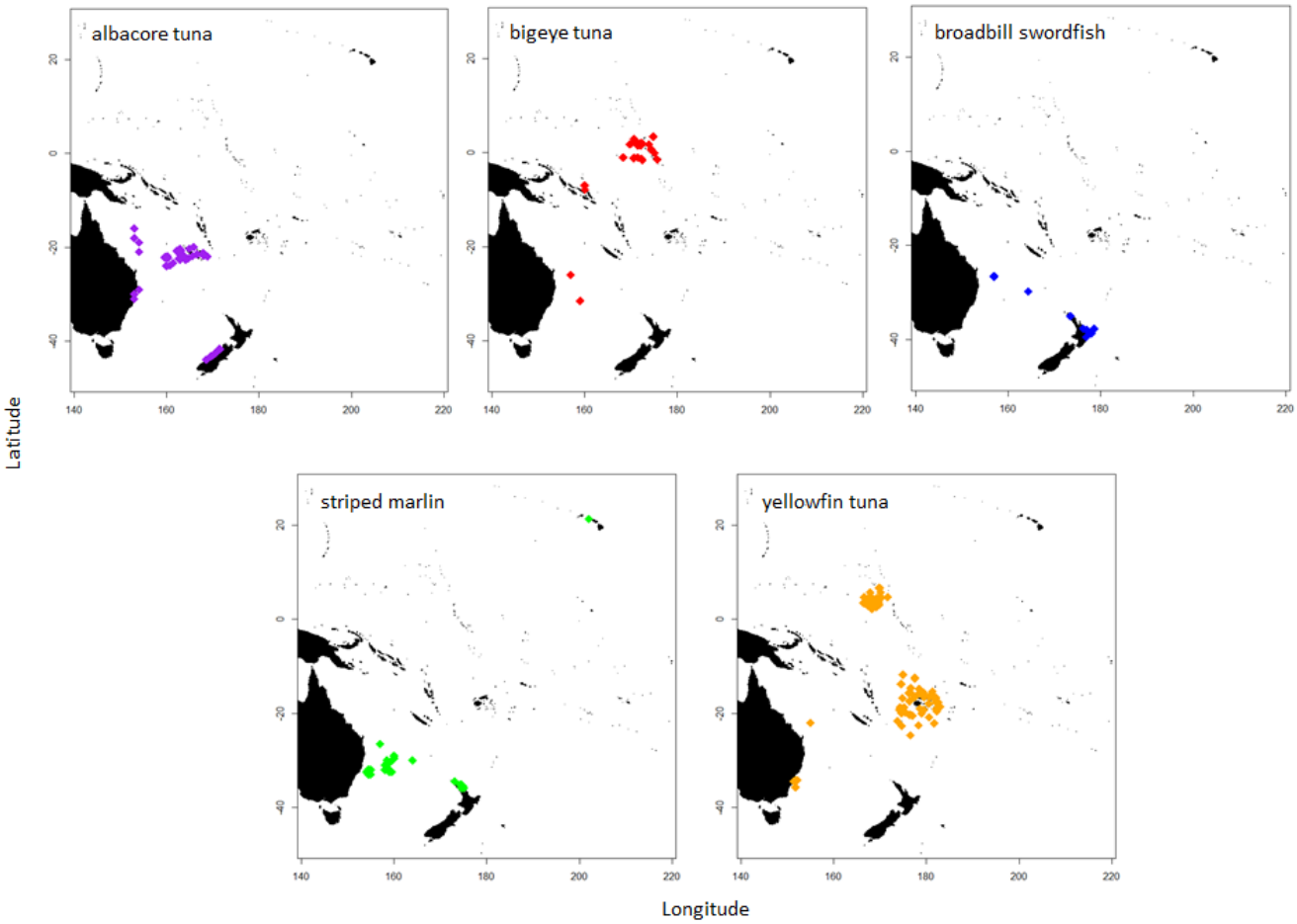

Figure 1. Sampling locations for albacore, bigeye and yellowfin tunas, broadbill swordfish and striped marlin.

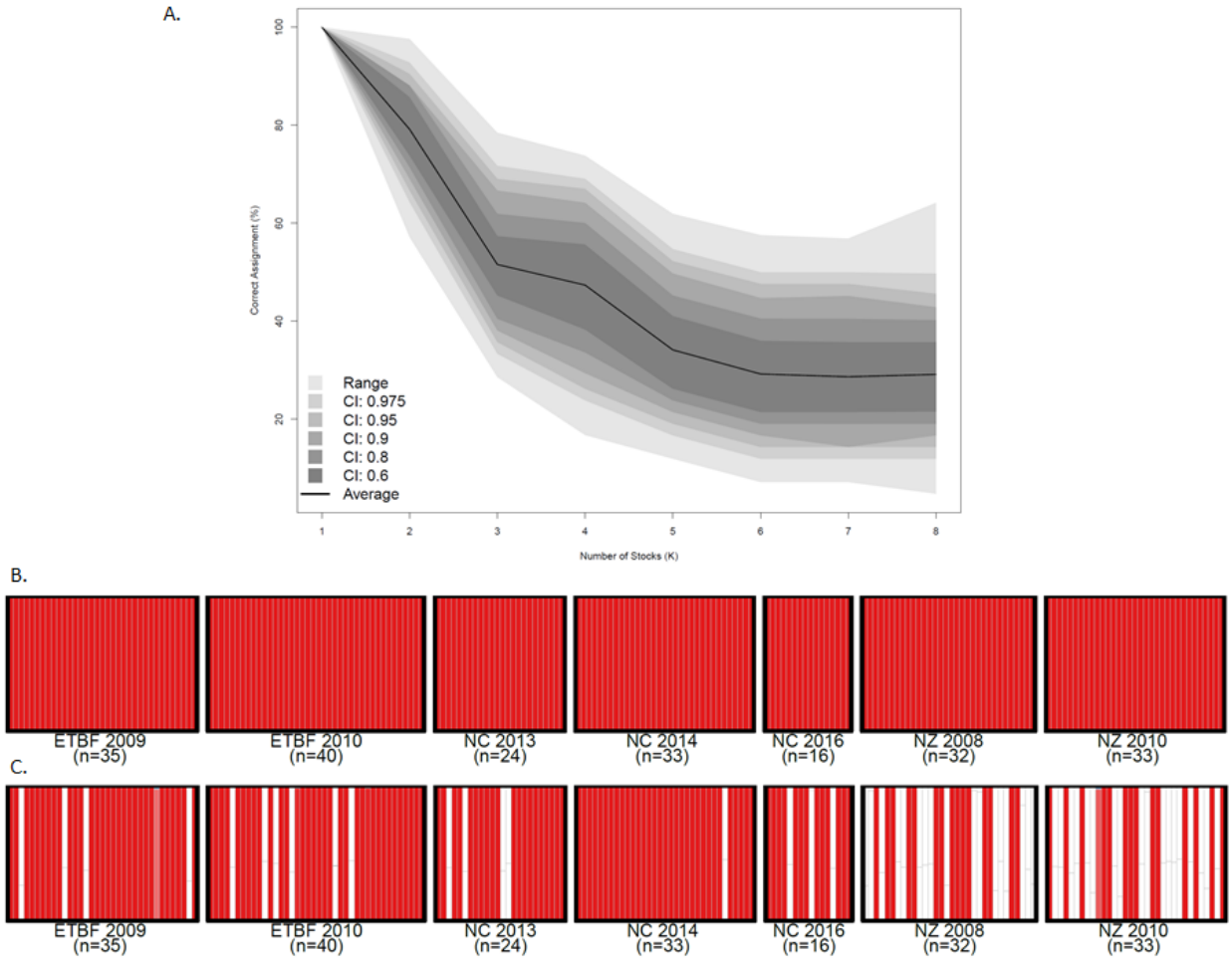

Figure 2. Output of the mixture model for albacore tuna. A. Cross validation and percent assignment of markers assuming K=1-8. B. The probability of individual assignment to K=1 and C. K=2. Weakening of colours (whiter) is reflective of the degree of uncertainty in assignment and where individuals are shaded in white the uncertainty of assignment is too high for the individual to be assigned to any of the groups in the scenario with any confidence.

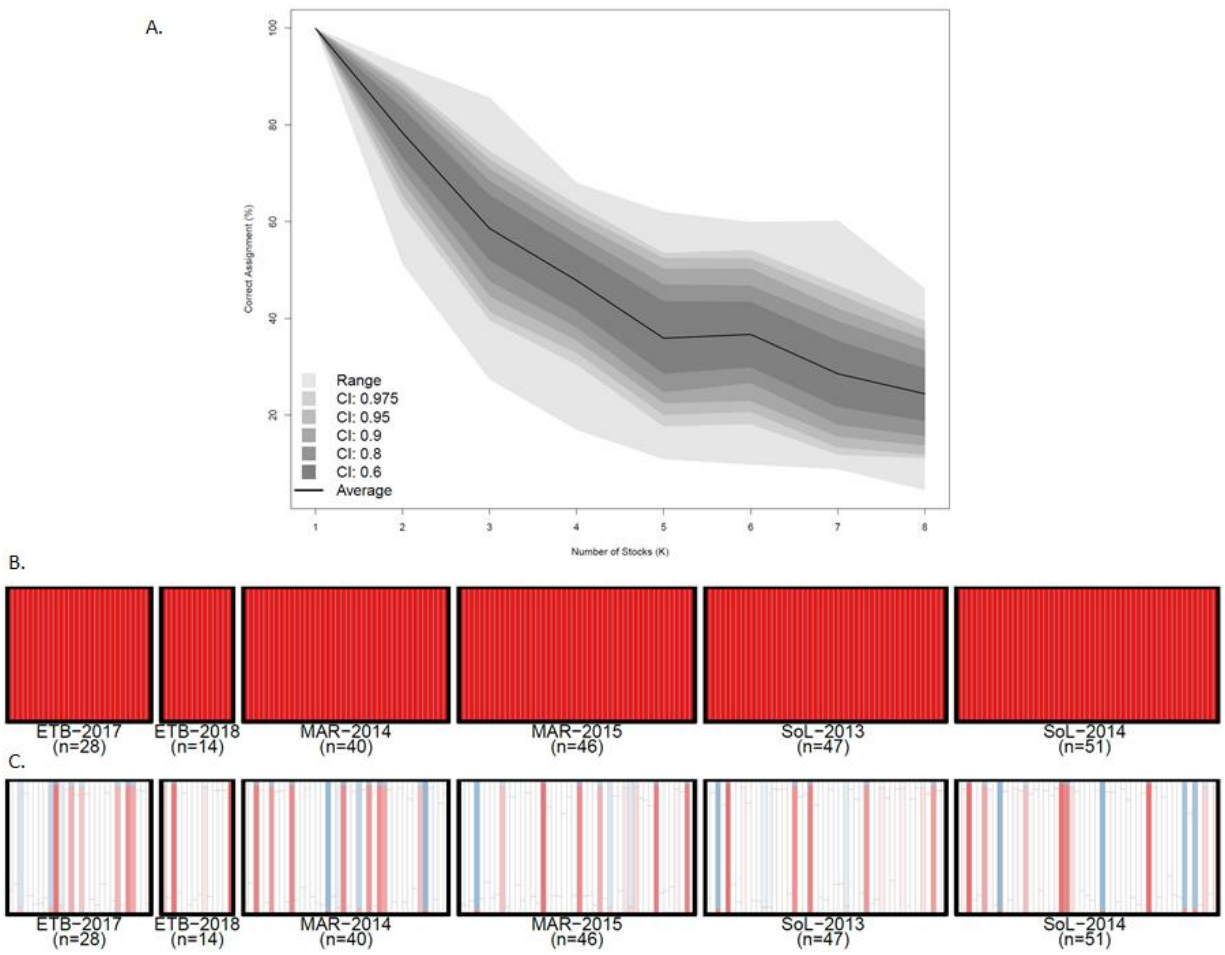

Figure 3. Output of the mixture model for bigeye tuna. A. Cross validation and percent assignment of markers on the basis of K=1 to K=8. B. The probability of individual assignment to K=1 and C. K=2. Weakening of colours (whiter) is reflective of the degree of uncertainty in assignment and where individuals are shaded in white the uncertainty of assignment is too high for the individual to be assigned to any of the groups in the scenario with any confidence.

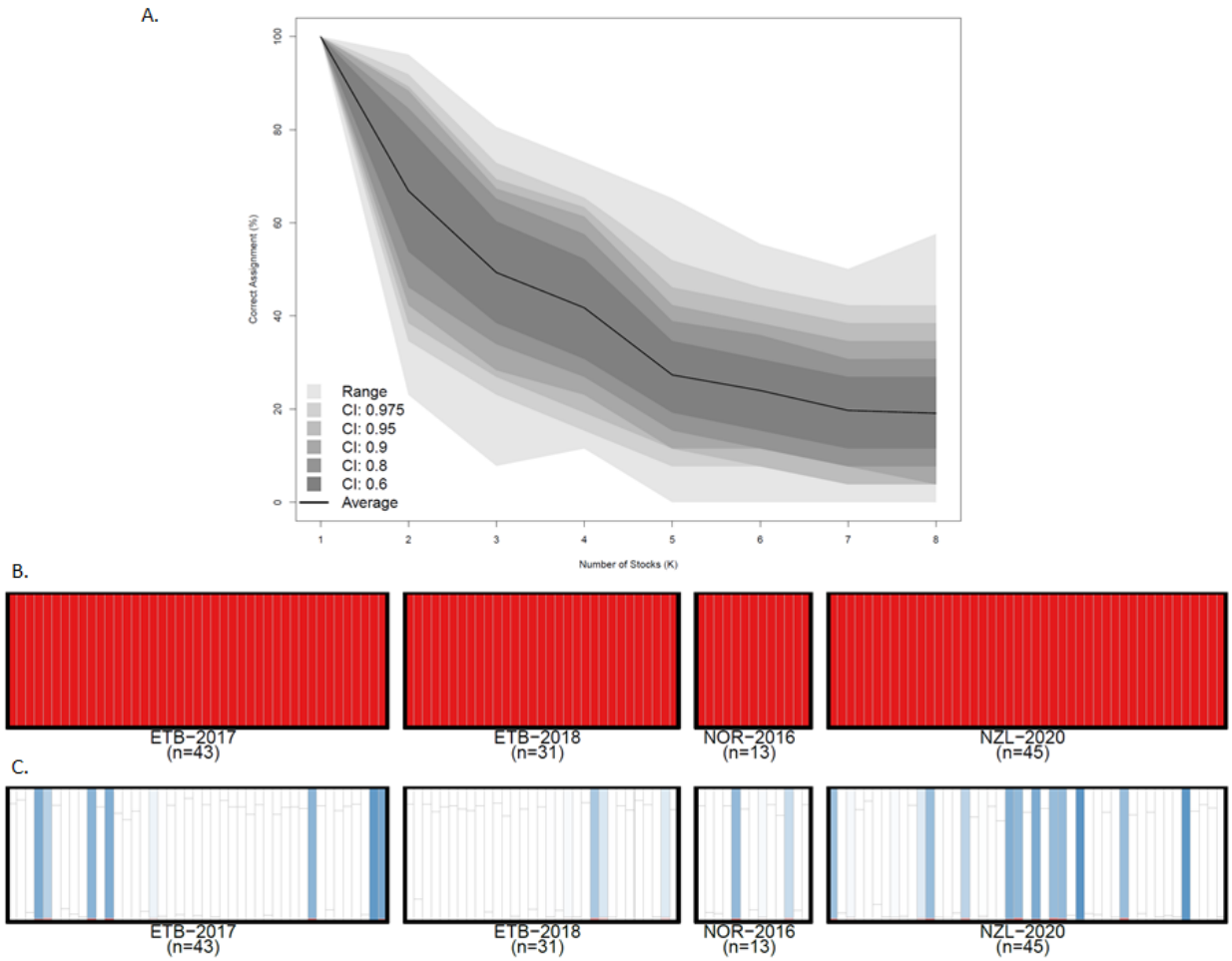

Figure 4. Output of the mixture model for broadbill swordfish. A. Cross validation and percent assignment of markers on the basis of K=1 to K=8. B. The probability of individual assignment to K=1 and C. K=2. Weakening of colours (whiter) is reflective of the degree of uncertainty in assignment and where individuals are shaded in white the uncertainty of assignment is too high for the individual to be assigned to any of the groups in the scenario with any confidence.

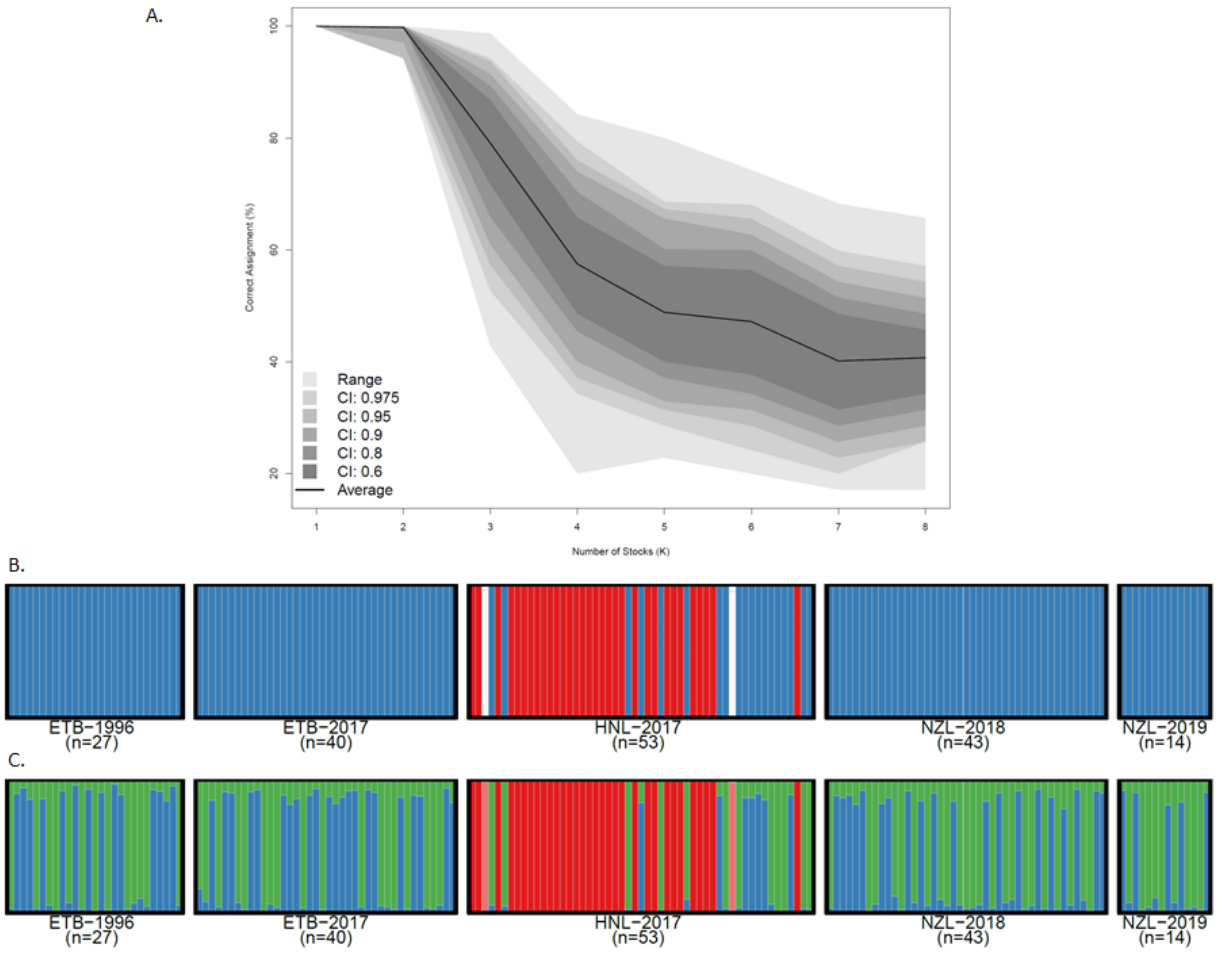

Figure 5. Output of the mixture model for striped marlin. A. Cross validation and percent assignment of markers on the basis of K=1 to K=8. B. The probability of individual assignment to K=2 and C. K=3. Weakening of colours (whiter) is reflective of the degree of uncertainty in assignment and where individuals are shaded in white the uncertainty of assignment is too high for the individual to be assigned to any of the groups in the scenario with any confidence.

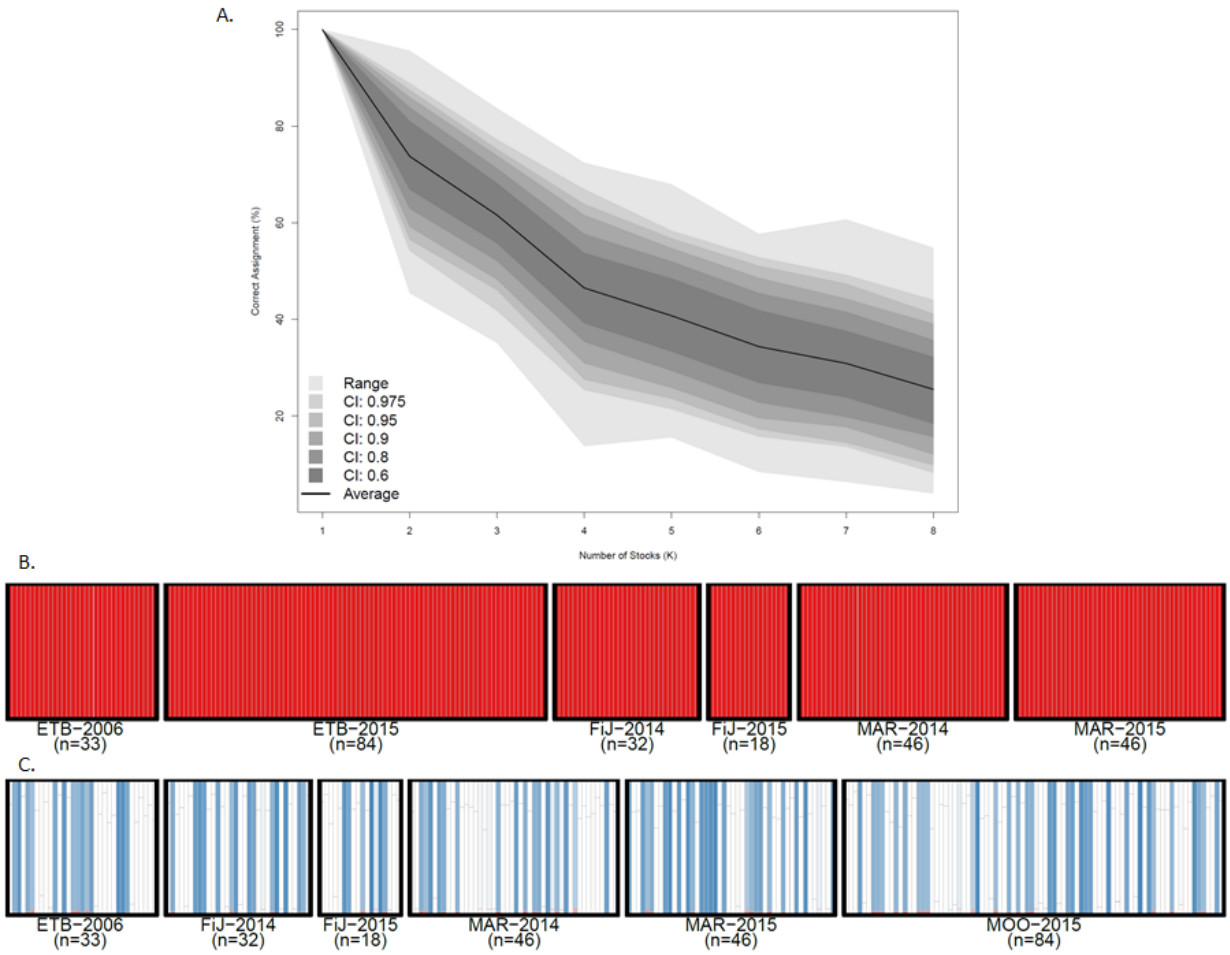

Figure 6. Output of the mixture model for yellowfin tuna. A. Cross validation and percent assignment of markers on the basis of K=1 to K=8. B. The probability of individual assignment to K=1 and C. K=2. Weakening of colours (whiter) is reflective of the degree of uncertainty in assignment and where individuals are shaded in white the uncertainty of assignment is too high for the individual to be assigned to any of the groups in the scenario with any confidence.