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Determination of the spatial dynamics and movement rates of the principal target species within the Eastern Tuna and Billfish Fishery and connectivity with the broader western and central Pacific Ocean - beyond tagging

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Abbreviations

AFMA: Australian Fisheries Management Authority

AIC: Akaike's Information Criterion

BIC: Bayesian Information Criterion

CSIRO: Commonwealth Scientific Industrial Research Organisation

DArT: Diversity Arrays Technology

DNA: Deoxyribonucleic Acid

EEZ: Exclusive Economic Zone

ETBF: Eastern Tuna and Billfish Fishery

FRDC: Fisheries Research and Development Corporation

PCR: Polymerase Chain Reaction

RFMO: Regional Fisheries Management Organisation

SNP: Single Nucleotide Polymorphism

TACC: Total Allowable Commercial Catch

TT-RAG: Tropical Tuna Resource Assessment Group

WCPFC: Western and Central Pacific Fisheries Commission

Executive Summary

What the report is about

This project was undertaken by CSIRO scientists that work on providing the tools and information required for undertaking one of the most complex forms of fisheries management: managing fish stocks at the national level when the species is part of a wider population that is shared by many fisheries and is managed by an international fisheries management organisation. Application of harvest strategies, required under Australia's Commonwealth Harvest Strategy Policy, to the tuna and billfish species caught in the Eastern Tuna and Billfish Fishery (ETBF) has been problematic, because uncertainty in the spatial connectivity of these species with the western Pacific Ocean is a key parameter affecting the performance of the current management approach. The work was conducted from 2016 to 2020 and involved employing next-generation genomic methods and cutting edge modelling approaches to investigate the connectivity of albacore, bigeye and yellowfin tunas and broadbill swordfish and striped marlin caught in the ETBF with the broader western and central Pacific Ocean. In doing so, this project also identifies what is needed to further reduce uncertainties in population structure relevant to harvest strategies and management frameworks for the ETBF.

Background

The ETBF operates in waters off the east coast of Australia and primarily targets albacore (*Thunnus alalunga*), bigeye (*T. obesus*) and yellowfin (*T. albacares*) tuna, broadbill swordfish (*Xiphius gladius*) and striped marlin (*Kajikia audax*). Populations of these species are considered to form part of at least a wider Western Pacific Ocean population with linkages between the ETBF and adjacent areas. Fisheries catches within the ETBF are managed under the Commonwealth Harvest Strategy Policy in line with regional management considerations under the auspices of the Western and Central Pacific Fisheries Commission (WCPFC). Stock assessments for individual species are conducted under the framework of the Commission and advice from stock assessments is considered in the formulation of suitable Conservation Management Measures (CMMs) such as limits on spatial operations, capacity restrictions, or catch levels for member states.

Currently, as part of WCPFC management and associated assessment processes, stocks of these five species are assessed as inter-connected single populations across the Western and Central Pacific Fishery Commission area. Although considered to comprise either one stock or two within each hemisphere under current regional management regimes, the degree of connectivity across the regions at which the species are assessed is still a major source of uncertainty. Traditional methods employed for investigating the connectivity and population structure of tuna and billfish species (conventional and electronic tagging, genetic approaches, analyses of fishery catches and biological approaches) have been unable to resolve uncertainties in the connectivity of these species, although all contain some spatial variability, suggesting that there may be some heterogeneity to stocks throughout the WCPFC area.

Application of harvest strategies, required under Australia's Commonwealth Harvest Strategy Policy, to the five principal species in the ETBF has been problematic, with uncertainty in spatial connectivity identified as a key parameter the performance of the current management approach. An assessment of the harvest strategy in relation to international fisheries concluded that reducing uncertainty in the spatial connectivity of species caught in the ETBF with the greater western Pacific Ocean was of particular priority. Without some knowledge of the identity and fidelity of individuals to spawning regions and the degree to which populations might be connected, management measures such as Total Allowable Commercial Catches (TACCs) set under domestic structures may not match regional management measures. This can result in unintended economic impacts on the domestic fishery if carried out independent of regional management.

Genetic methods have undergone a technical revolution in the last decade and in association, can now achieve much higher resolution than was possible in the past. Genomic approaches now offer solutions to a number of problems in fisheries management, allowing identification of fish at the level of (i) species, (ii) genetic grouping, and (iii) individual. A feasibility study conducted by CSIRO and AFMA investigated the potential use of next generation genomic approaches in reducing uncertainty in the stock structure of the tropical tuna species. The study concluded that of all methods likely to provide information on the stock structure and connectivity of species, molecular methods are most likely to be (i) cost effective; (ii) logistically feasible across the western and central Pacific Ocean and (iii) most likely to robustly be able to provide insights into any spatial structure in populations.

Aims/objectives

1. Investigate the presence of stock structure in the five principal species caught in Australia's Eastern Tuna and Billfish Fishery and the western Pacific Ocean across spatial scales of relevance using new generation genomic methods;

2. Assess the need and associated costs for research required to further reduce uncertainties in stock structure relevant harvest strategies and management frameworks;

3. Inform the relevant parties in the Western and Central Pacific Fisheries Commission of the key results and, if appropriate, the need and value of extending the project throughout the western and central Pacific Ocean.

Methodology

Samples of each of the five species were collected from three sample sites, one in the ETBF and two sites external to the ETBF in the western Pacific Ocean. Samples were collected from these three sites during two sampling periods separated by at least 12 months (i.e. two temporal events across each of the three sites for each species). Sampling across two temporal events represents the minimum temporal replication that was feasible within the project budget and timeframe. Sites external to the ETBF were chosen on the basis of feasibility of collection of samples (where new sampling was undertaken) or availability of sufficient numbers of samples (where archived samples were utilised). After preparation and extraction of DNA at CSIRO, extracts were shipped to Diversity Array Technologies in Canberra where approximately 2,000,000 sequences per barcode/sample were identified. These sequences were then analysed to identify single nucleotide polymorphism (SNP) variation and to deliver a dataset of co-dominant SNP-DArT genotypes used for downstream population genetic analysis. Species identification was then confirmed, and sequenced datasets were quality control filtered. Population modelling using a mixture model provided a probability of assignment of each sample to each of K groups with a distinct genetic profile and provided information about how many genetic groups there were likely to be amongst samples. Model summaries were then used to estimate the chance of each sample belonging to each genetic group.

Results/key findings

Accessing samples from broadbill swordfish from two sites within the WCPFC area was particularly problematic and was exacerbated by a poor fishing season in 2019. This resulted in samples for broadbill swordfish consisting of samples collected from the ETBF (2 years), Norfolk Island (1 year) and New Zealand (1 year). The poor fishing season in 2019 also resulted in limited samples of striped marlin from New Zealand being collected in the second year of samples. The genetic groupings identified across bigeye and yellowfin tunas and broadbill swordfish suggest a substantial level of connectivity and mixing between each of the locations investigated, with little discernible genetic differentiation between areas. Results from albacore suggest the potential for two genetic groupings, however these were not able to be resolved by the methods used. 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 group was identified only from samples collected from Hawai'i. The presence of two genetic populations of striped marlin in the waters of Hawai'i has been proposed previously and the results presented here lend further support to this

hypothesis. The consistent absence in the ETBF and New Zealand of the second genetic group found in Hawai'i indicates a proportion of fish recruiting to the Hawai'i fishery do not contribute to the ETBF fishery and potentially represent a northern hemisphere population that doesn't migrate south of the equator.

The results of the current study are largely consistent with previous genetic investigations into the population structure of these four species. Consistency in results across years suggest that the groupings revealed here have some temporal stability across years across those sites where multiple years of samples were collected. Although results suggest the potential for two genetic groupings among albacore samples, assignment by the methods used here was statistically uncertain and resulted in some individuals not being able to be assigned to either group in the scenario with any confidence. Further sampling from the three locations included here as well as inclusion of samples from additional sites would also be required for resolving these uncertainties.

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 with any certainty. Further sampling and analysis of sites across the western and central Pacific, including temporal replication of sampling, would be needed to investigate whether the results presented here are consistent with other locations across the western and central Pacific region or whether greater genetic differentiation is discernibly present. The resources required to support the attainment of broader insights into the connectivity of species across the WCPFC Area and connectivity between the ETBF and the western and Central Pacific Ocean will be dependent on current access to samples, the extent of further sampling required in order to attain broad spatial and temporal coverage of samples, the facilities and capability available for processing and sequencing samples and the capability available for data quality control and analysis pipelines.

As next steps, a second year of sampling for broadbill swordfish from New Zealand is planned and a preliminary small dataset from the Cook Islands (consisting of 24 samples) has been collected. These samples will be analysed and incorporated with the data from this project to provide further insights into the connectivity of broadbill swordfish across the western and central Pacific Ocean and presented to the WCPFC Scientific Committee in August 2021.

Implications for relevant stakeholders

The results of this project 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. Results from the project lend support to current assessment structures undertaken across the WCPFC Area and provide confidence in ensuring that management measures set under domestic structures align with regional management measures.

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. 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 importantly some temporal duplication of sampling is required. Care also needs to be taken in the quality control of samples and datasets and choice of analytical methods used to reduce uncertainties in results. This will require dedicated efforts placed towards the development, design and carrying out of such a program.

Recommendations

The population structure of tuna and billfish species across the western and Central Pacific Ocean is of increasing interest given the potential of genomics using SNPs in resolving questions around structuring and connectivity. Key recommendations from this project are:

1. To work nationally and with regional partners within the WCPFC to collect and analyse samples from a larger set of locations to better understand the population structure and connectivity of target species caught within the western and central Pacific Ocean. Further to work with regional partners more broadly to understand population structure at basin scales and to better understand the connectivity of species between regional fisheries management organisation areas.
2. To continue to work closely with the Tropical Tuna Resource Assessment Group and researchers developing harvest strategies for ETBF species to provide updated information on the connectivity of species caught in the ETBF with those caught in the WCPFC area and using that information, engage where relevant in developing harvest strategies more broadly across the western and central Pacific Ocean.
3. To continue to engage with the Pacific Community and the WCPFC to provide information relevant for further developing sampling programs for the WCPFC Tropical Tuna Tissue Bank that can support population structure studies.

Keywords

Population structure, Western and Central Pacific Ocean, albacore tuna, bigeye tuna, broadbill swordfish, yellowfin tuna, striped marlin.

Introduction

Background

Commercial fisheries within Australia target several pelagic species that have broad distributions at the scale of ocean basins. Fisheries targeting these species comprise multiple fleets from a number of nations that fish within and outside country EEZs across the range of these species. Because of the extensive distributions of pelagic species harvested and the associated distribution of the fleets that target them, management of these species often occur at both the national and regional level. Within Australia, there are five fisheries associated with pelagic species that are managed at multiple scales; these include the Southern Bluefin Tuna Fishery, the Eastern Tuna and Billfish Fishery (ETBF), Western Tuna and Billfish Fishery, the Eastern Skipjack Tuna Fishery and the Western Skipjack Tuna Fishery. Management of these fisheries occurs at the national level within the Commonwealth Harvest Strategy framework (except southern bluefin tuna which is managed under the Southern Bluefin Tuna Fishery Management Plan) and at the international level by RFMOs.

The ETBF operates in waters off the east coast of Australia and primarily targets albacore (*Thunnus alalunga*), bigeye (*T. obesus*) and yellowfin (*T. albacares*) tuna, broadbill swordfish (*Xiphius gladius*) and striped marlin (*Kajikia audax*). Populations of these species are considered to form part of at least a wider Western Pacific Ocean population with linkages between the ETBF and adjacent areas. Fisheries catches within the ETBF are managed under the Commonwealth Harvest Strategy Policy in line with regional management considerations under the auspices of the Western and Central Pacific Fisheries Commission (WCPFC). Stock assessments for individual species are conducted under the framework of the Commission and advice from stock assessments is considered in the formulation of suitable Conservation Management Measures (CMMs) such as limits on spatial operations, capacity restrictions, or catch levels for member states.

Currently, as part of WCPFC management and associated assessment processes, stocks of these five species are assessed as inter-connected single populations across the western and central Pacific commission Area (McKechnie et al. 2017; Tremblay-Boyer et al. 2017; Takeuchi et al. 2017; Tremblay-Boyer et al. 2018; Ducharme-Barth et al. 2019). Although considered to comprise either one stock or two within each hemisphere under current regional management regimes, the degree of connectivity across the regions at which the species are assessed is still a major source of uncertainty (Gunn et al. 2002; Gunn et al. 2005; Itano et al. 2008; Nikolic and Bourjea 2013; Evans et al. 2014; Evans et al. 2015; Evans et al. 2016; Moore et al. 2020a).

Stock assessments for albacore, bigeye and yellowfin tunas throughout the western and central Pacific Ocean assume that mixing of tagged and untagged individuals within an assessment region happens relatively quickly, and that all individuals of a given age have the same probability of moving among regions, irrespective of their individual histories (McKechnie et al. 2017; Tremblay-Boyer et al. 2017; Takeuchi et al. 2017; Tremblay-Boyer et al. 2018; Ducharme-Barth et al. 2019). An investigation into the mixing rates of tags deployed on bigeye and yellowfin tunas found strong evidence for incomplete mixing after one quarter for bigeye tuna and after five quarters for yellowfin tuna (Kolody and Hoyle 2013). Estimated periods of incomplete mixing were regarded as minimums largely because observations (tags at liberty for extended periods of time) restricted the ability to make inferences on mixing in relation to longer periods at liberty (Kolody and Hoyle 2013). This analysis suggested that rates of mixing used in stock assessments for these species may not be appropriate and would be expected to bias assessment estimates of mortality, abundance and movement. Although tagging data and the results from molecular analysis of striped marlin suggest the presence of a semi-independent stock in the south west Pacific Ocean, movements of the species are considered large enough that there is a relatively high level of regional mixing. As a result, the most

recent stock assessment for striped marlin in the WCPFC area incorporates a single model region (Ducharme-Bath et al. 2019). Similarly, although tagging data for broadbill swordfish suggest limited mixing particularly in the south-west Pacific Ocean, the most recent stock assessment incorporates a single model region with instantaneous and complete mixing of the population (Takeuchi et al. 2017). Assessments remain sensitive to assumptions associated with tagging data, including assumed mixing periods. Estimation of more definitive rates of movement across regions in the western and central Pacific Ocean is desirable for all five species.

Connectivity of tuna and billfish species

Individuals of bigeye and yellowfin tuna, broadbill swordfish and striped marlin caught and tagged with conventional and electronic tags within the ETBF have been observed to move beyond the eastern limits of the ETBF (Hampton and Gunn 1998; Evans et al. 2008; Domeier 2006; Evans et al. 2014). In some cases, individuals tagged outside of the Australian Exclusive Economic Zone (EEZ) have been observed to move into areas just adjacent to, or into the eastern parts of the ETBF (Domeier 2006; Sippel et al. 2011; Hillary and Patterson 2019). Although movements of bigeye and yellowfin tuna, broadbill swordfish and striped marlin into and out of the ETBF have been recorded, most observations suggest that localised residency on scales smaller than the ETBF may occur (e.g. Evans et al. 2011). Small sample sizes, a focus on tagging particular life stages of some species, limited deployment periods and the coarse resolution of position estimates derived via geolocation, particularly those associated with latitude (see Evans and Arnould 2009), restrict the ability to determine the overall spatial dynamics of species at these scales. As a result, it is unknown if such residency behaviours occur over longer time scales and across all life stages. To date there have been no long-term large-scale tagging programs on albacore due to low recovery rates and short tag retention times (Williams et al. 2015). However longline fishery catches suggest albacore migrate seasonally between tropical and subtropical waters corresponding with shifts in the 23 - 28° C sea surface temperature isotherm (Langley 2004).

Identification of specific spawning areas throughout the western Pacific Ocean, the degree of fidelity to spawning sites and the origin of recruits within the ETBF are still largely unknown for all five species. Aggregations of bigeye and yellowfin tunas in the northern Coral Sea have been associated with spawning (McPherson 1988). Examination of the gonads of female yellowfin tuna from the northern Coral Sea region of the ETBF documented advanced stages of oocyte maturation, suggesting individuals were spawning in the region during the months of October and November (McPherson 1991). Actively spawning albacore tuna have been recorded from the northern Coral Sea across the months October to December (Farley et al. 2013). Scombrid larvae have been observed in plankton tows in the northern waters of the Great Barrier Reef (Leis and Goldman 1984). Although not identified to species by Leis and Goldman (1984), larval tows conducted by others have identified larvae from albacore, yellowfin and bigeye tunas with densities of larvae highest across October – December (Nishikawa et al. 1985).

Preliminary analysis of the microchemistry of yellowfin tuna otoliths support the hypotheses that the Coral Sea region is a major source of recruits to the fishery within the ETBF, but that there are also linkages with Indonesia and the Solomon Islands (Gunn et al. 2002). Further analyses to establish the degree to which yellowfin tuna recruits are sourced from within the Coral Sea region as opposed to recruits from outside the Coral Sea and their ongoing residency within the Coral Sea has not been undertaken. Across the broader western and central Pacific Ocean, these techniques have shown some promise in discriminating a degree of spatial structure in both bigeye and yellowfin tunas (Wells et al. 2012; WPRFMC 2014). However, the method at this point in time is unable to determine if species with similar chemical signatures are derived from larval pools that freely mix or from larval pools that may not mix, but occur in areas of similar water chemistry (McDonald et al. 2013). Determining which situation occurs requires not only analysing otoliths from individuals across the

species' range, but also water from spawning regions. This increases the effort and cost required for such investigations to robustly examine questions associated with stock structure.

Genetic studies conducted on the main species caught within the region have primarily focused on determining the level of genetic structure at the global level, comparing genetic structure between ocean basins (Ward et al. 1997; Alvarado Bremer et al. 1998; Graves and McDowell 2003; Viñas et al. 2004; Bradman et al. 2011; Montes et al. 2012; Laconcha et al. 2015; Mamoozadeh et al. 2020). Those that have investigated potential genetic structure associated with the presence of defined spawning populations within the Pacific Ocean basin have historically been inconclusive and generally limited by small sample sizes (Scoles and Graves 1993; Ward et al. 1994; Rosel and Block 1996; Grewe and Hampton 1998; Reeb et al. 2000; Appleyard et al. 2001; Chiang et al. 2006). The inconclusive nature of results could be the result of a number of factors. First, only a small amount of gene flow (a few migrants per generation) may be sufficient to obscure genetic differentiation between conspecific stocks (Hauser and Ward 1998). Second, connectivity might be largely facilitated by larval dispersal, with larvae transported regularly between regional and distant populations (Cowen et al. 2007; Cowen and Sponaugle 2009), information that is largely unknown. Third, given the large population sizes and subtle nature of population differentiation observed for marine species, the molecular markers investigated to date may have lacked sufficient resolution to differentiate stocks that exhibit only a small degree of isolation and finally, sample sizes required to resolve any structure may have been inadequate (Kasapidis et al. 2008; Bradman et al., 2011; Grewe et al., 2015).

Commercial catch data, biological information on growth rates and reproduction, and initial investigations of hard part chemical signatures all demonstrate some spatial variability. This spatial variability across datasets suggests that there may be some heterogeneity to stocks throughout the WCPFC area that has not been able to be resolved via traditional genetic approaches (Hillary et al. 2016).

Implementation of harvest strategies for the ETBF

The harvest strategy developed for the ETBF aims to provide a means by which assessments of albacore, bigeye and yellowfin tunas, broadbill swordfish and striped marlin can determine a Recommended Biological Commercial Catch (RBCC). The RBCC is then used to set a Total Allowable Commercial Catch (TACC) for each of the species. The simulation-tested harvest strategy should be robust to the main assessment uncertainties, and aims to provide for rebuilding of overfished stocks, enable sustainable development of emerging fisheries and reduce inter-annual variability in TACCs, thereby providing stability to the fishing industry. The decision rule framework for the harvest strategy originally identified that it should be "robust" to uncertainty about linkages between regional and broader western and central Pacific Ocean stocks and should respond to declines and increases in regional stock status, regardless of whether they are generated by domestic or international fleets (Davies et al. 2007). However, at the time of development, it was beyond the scope of the project developing the harvest strategy to evaluate all species, or to examine approaches for incorporating spatial linkages between domestic and broader international fisheries.

The harvest strategy developed was used to inform the setting of total allowable effort for the 2009-11 fishing seasons in the ETBF. Following the development of the framework, comprehensive evaluation in order to produce fully specified harvest strategies using a detailed management strategy evaluation (MSE) framework was undertaken (Kolody et al. 2010). Across all five species, the harvest strategy framework demonstrated sensitivity to population connectivity, the effects of the non-ETBF fleet (the wider western and central Pacific Ocean fleet) on the overall population and lack of agreement between catch per unit effort time series for domestic fleets with that used in broader stock assessments (Kolody et al. 2010). In the case of bigeye and yellowfin tunas, it was concluded that the ETBF is likely to have little effect on regional populations, resulting in the harvest strategy being disconnected from the basic feedback principle on which it was developed (Kolody et al. 2010).

The preliminary nature of regional stock assessments for both albacore tuna and striped marlin, which were used in the harvest strategy, also resulted in substantive uncertainty in harvest strategy outputs. As a result of the sensitivities of the harvest strategy framework and resulting unsuitability for use on the three tuna species, the framework has only been used to set TACCs for broadbill swordfish and striped marlin. Total allowable catches for the three tuna species have been set based on historical catch levels. Further exploration of the harvest strategy through the development of updated operating models for bigeye and yellowfin tuna found that there was little to no feedback in the harvest strategy system and a lack of substantive enough data to be able assess whether the harvest strategy would illicit feedback (Hillary et al. 2016).

Application of the harvest strategy to the five principal species has continued to be problematic, with uncertainty in spatial connectivity identified as a key parameter the performance of the current management approach. An assessment of the harvest strategy in relation to international fisheries concluded that reducing uncertainty in the spatial connectivity of species caught in the ETBF with the greater western Pacific Ocean was of particular priority and that the most cost effective and appropriate methods for reducing uncertainty were next generation genomic methods and associated analysis methods (Hillary et al. 2016).

Next generation genetic approaches to resolving stock structure

Genetic methods have undergone a technical revolution in the last decade. Genomic approaches now offer solutions to a number of problems in fisheries management, allowing identification of fish at the level of (i) species, (ii) genetic grouping, and (iii) individual (Grewe et al. 2015; Bravington et al. 2016; Kappel et al. 2017; Proctor et al. 2019). In addition, these methods are now much more cost effective than they have previously been. The advantage is that they provide a powerful low cost method for high through-put analysis of individuals, thereby allowing investigation of questions associated with stock structure, stock abundance, traceability of products and identification of species at cost-competitive rates (van Dijk et al. 2014). This makes these approaches very cost competitive in comparison to conventional monitoring and assessment methods (e.g. conventional tagging, traditional surveys).

While genetic studies have had very limited success demonstrating fine-scale population genetic structure of pelagic marine species, next generation genomic methods have begun to resolve structure in populations. Evidence of population structure at sub-basin scales has recently been demonstrated in both yellowfin tuna and striped marlin (e.g. Grewe et al. 2015; Mamoozadeh et al. 2020). A feasibility study conducted by CSIRO and AFMA investigated the potential use of next generation genomic approaches in reducing uncertainty in the stock structure of the tropical tuna species(Evans et al. 2016). The study concluded that of all methods likely to provide information on the stock structure and connectivity of species, molecular methods are most likely to be (i) cost effective; (ii) logistically feasible across the western and central Pacific Ocean and (iii) most likely to robustly be able to provide insights into any spatial structure in populations. The study concluded that if the application of such methods revealed a revised and more detailed understanding of population structure, close-kin mark-recapture estimators and feasible sampling regimes could be developed. Once implemented, these should further reduce uncertainty in spawning stock size, and could potentially provide an abundance index for the harvest strategy that reduces reliance on commercial catch rates as relative abundance indices.

Need

Management of the ETBF is complex because of the cross-jurisdictional nature of the stocks and governance through the Commonwealth Harvest Strategy Policy and the WCPFC. Current assessments conducted by the WCPFC assume that these species comprise either single discrete

stock units throughout the WCPFC area or across the Southern Hemisphere portion of the region and genetic methods used in the past have been unable to refute such assumptions. Biological information on growth rates and reproduction, movement data derived from tagging studies and spatial and temporal variability in catches of these species, however, suggest that there is likely to be some structure to stocks throughout the WCPFC region and assumptions of single spawning populations may not be accurate.

If any of the principal species occurring in the ETBF do comprise localised stocks, this has obvious implications for the management both within national and regional contexts. Without some knowledge of the degree of identity and fidelity of regions of spawning and populations might be connected, management measures such as TACCs set under domestic structures may not match regional management measures. This can result in unintended economic impacts on the domestic fishery if carried out independent of regional management (DAFF 2013).

Clarification of the connectivity and population structure of species in Australia's Tropical Tuna fisheries with the broader WCPFC region is required for appropriate governance through the Commonwealth Harvest Strategy Policy and the WCPFC, to ensure any risks to regional stock biomass are minimised and to improve stakeholder concern over stock management.

In 2015, FRDC requested expressions of interest for projects to determine whether the Australian principal target species within the ETBF are a separate stock from that of the broader WPO based on modern techniques that are more discriminatory than previous stock identity methods. This project has been developed to address this research priority and key aspects of this project have been formulated based on attendance at Tropical Tuna Resource Assessment Group (TT-RAG) meetings, interaction and discussion with key stakeholders, end-users and potential beneficiaries of the project material at various management fora.

Of relevance in facilitating such a study, has been the concurrent development of the WCPFC Tropical Tuna Tissue Bank. The tissue bank has been partnering with fisheries observer programmes operating in the western and central Pacific Ocean in order to provide a collection of biological samples of pelagic species from all over the Pacific Islands region on behalf of its member countries. The tissue bank aims to collect approximately 2,000 samples for each species in order to allow Pacific-wide studies to be undertaken. The tissue bank is now at the stage where such studies can be facilitated. Notably this project provided an opportunity to evaluate the WCPFC Tropical Tuna Tissue Bank in relation to its ability to support investigations of the stock structure of tuna species across the western and central Pacific Ocean. In undertaking such an evaluation, useful guidance can be provided to the WCPFC for planning future development of the Tropical Tuna Tissue Bank.

Over the course of this project, the need for resolving uncertainties associated with connectivity has become more pertinent, with work underway revising the harvest strategies for broadbill swordfish and striped marlin in the ETBF. These revisions are needed because the current harvest strategies do not perform satisfactorily under current configurations and scenarios. As such a key requirement of the harvest strategies is to establish the extent of mixing across the western and central Pacific Ocean and incorporate a level of potential population structure into operating models used to structure the harvest strategies.

At the same time there has been growing recognition that uncertainties surrounding the stock structure of the four tuna species targeted across the WCPFC area could have important impacts on population dynamics models used to assess stock status and inform management options by the WCPFC. In 2018 a regional workshop was held with the aim of identifying current understanding of the spatial dynamics and connectivity of albacore, bigeye, skipjack and yellowfin tunas and what would be needed to be undertaken as a body of work in order to resolve the stock structure of the four species (Moore et al. 2020a; 2020b). This was followed up with a second workshop involving

Pacific Community, CSIRO, ABARES and AFMA staff where the development of a potential collaborative work program that might start to address uncertainties in connectivity and stock structure was discussed.

Objectives

Objective Number	Objective Description
1	Investigate the presence of stock structure in the five principal species caught in Australia's Eastern Tuna and Billfish Fishery and the western Pacific Ocean across spatial scales of relevance using new generation genomic methods
2	Assess the need and associated costs for research required to further reduce uncertainties in stock structure relevant harvest strategies and management frameworks
3	Inform the relevant parties in the Western and Central Pacific Fisheries Commission of the key results and, if appropriate, the need and value of extending the project throughout the western and central Pacific Ocean.

Method

Sample collection

A full inventory of historical samples of muscle tissue held by CSIRO was conducted with potential samples from the five key target species available for use identified. A spatial assessment of tissue samples for tropical tuna and billfish species held in the WCPFC Tropical Tuna Tissue Bank and key areas where samples from albacore, bigeye and yellowfin tuna were available for stock structure analyses identified.

As a first step in investigating the viability of those historical muscle tissue samples identified and held by CSIRO and in the WCPFC Tuna Tissue Bank, a quality control sequencing trial of samples was run in late 2016/early 2017. This involved sub-sampling five muscle tissue samples from each of the five species from each of the two collections (for a total of ten samples per species), extracting the DNA from each sample and shipping the DNA aliquots to DArT for gene sequencing (see below for details of methods). All samples from the WCPFC Tuna Tissue Bank passed DArTSeq™ quality control checks and overall results from CSIRO collections were also positive, with the majority passing quality control checks. These results provided the project with the confidence that utilisation of historical collections was possible.

The design that was targeted for the project consisted of a minimum of 50 muscle tissue samples in each of two sampling events separated by 12 months from three spatially restricted (i.e. minimising the dispersal of samples across large regions) locations for each species. The three locations comprised the ETBF and two sites within the western Pacific Ocean.

Potential sites external to the ETBF were discussed with relevant Pacific Community (SPC) staff managing the WCPFC Tropical Tuna Tissue Bank within the context of access to adequate numbers of muscle tissue samples. Two applications for access to muscle tissue samples from albacore, bigeye and yellowfin tunas held in the WCPFC Tuna Tissue Bank were approved by the WCPFC Secretariat. A second application was required as a result of sampling discrepancies identified by SPC staff and an associated need to change the locations of some samples from those set out under the original agreement. Individual samples of muscle tissue were extracted from the WCPFC Tropical Tuna Tissue Bank, with approximately 1gm of tissue sub-sampled and placed into individual vials of RNALater. Samples were refrigerated for 24 hours and then shipped to the CSIRO laboratories with associated metadata detailing capture information. Samples were then archived until further preparation.

Where muscle tissue samples held in either the CSIRO or the WCPFC Tropical Tuna Tissue Bank collections did not meet the experimental design requirements of the project, the feasibility of further sampling to resolve spatial gaps and/or inadequate numbers was explored through a number of avenues including with ETBF fishers, Pacific Community staff, WCPFC member representatives, the TT-RAG, within country fisheries agency staff, the Western Pacific Regional Management Council and fisheries consultants operating across the western and central Pacific Ocean.

Within the ETBF, collection of additional muscle tissue samples to those held in CSIRO archives from bigeye tuna, broadbill swordfish and striped marlin was conducted via sampling of fish during onshore processing either at De Brett Seafoods or Walker Seafoods Australia based in Mooloolaba, Queensland. External to the ETBF, project collaborators including staff from Bluewater Marine and Bay Packers (NZ) Ltd in New Zealand collected samples from broadbill swordfish and striped marlin, staff from the Western Pacific Regional Fishery Management Council and University of Massachusetts collected samples from striped marlin and staff from the Ministry of Marine Resources, Cook Islands collected samples from broadbill swordfish on behalf of CSIRO as part of

routine operations. A unique opportunity to collect samples from broadbill swordfish caught in the Norfolk Island fishery in 2016 was made available to the project.

Regarding sampling from the ETBF, the Cook Islands and New Zealand, samples of approximately 1gm of muscle tissue were either directly sampled from individual fish or sub sampled from larger samples and placed into individual vials of RNALater. Sample vials were then refrigerated for a minimum of 24 hours prior to being shipped to the CSIRO laboratories. In the case of samples from Hawai'i, tissue attached to fin spines were placed in a solution of EDTA saturated with NaCl and then refrigerated prior to being shipped. All samples were archived the CSIRO laboratories until further preparation.

DNA extraction

For DNA extraction, muscle tissues were firstly cleaned by removing all external surfaces of each muscle tissue sample using a new scalpel blade for each individual. This removed potentially surface contaminated tissue from the sample, with use of a new scalpel blade per sample avoiding any further surface cross contamination of samples. Total genomic DNA was isolated from the cleaned tissue samples (approximately 15 mg in weight) using one of two protocols; either (i) a Machery Nagel Nucleo-Mag bead-based DNA isolation kit or (ii) a C-TAB 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. Five uL aliquots of each extract were visually inspected using gel electrophoresis as a first-pass qualitative check of the quality of the DNA in each sample. Samples that were qualitatively assessed as containing inadequate (<1ng/uL) amounts of DNA or highly degraded DNA were removed and did not progress to sequencing.

Genetic sequencing

DNA extracts 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 minute followed by 30 cycles of 94°C for 20 seconds, 58°C for 30 seconds and 72°C for 45 seconds, with a final extension step at 72°C for 7 minutes. 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. DArTseq PL's proprietary DArT-Soft14 pipeline analysed these sequences to identify single nucleotide polymorphism (SNP) variation and to deliver a dataset of co-dominant SNP-DArT genotypes used for downstream additional quality control pipelines and population analysis.

Species identification

As a first step in quality control processes, the species of all samples was confirmed. Identification of swordfish, and striped marlin were confirmed using polymerase chain reaction – restriction fragment length polymorphisms (PCR-RFLP) analysis of a 1400bp region of the mitochondrial DBA molecule as described in Innes et al. (1998). Identification of albacore, bigeye, and yellowfin tuna were confirmed following restriction digestion of PCR-RFLP as described by Chow and Inoue (1993) with further modifications described by Takayama et al. (2001).

Size specific banding patterns representing the resulting 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 R package RADIATOR (Gosselin 2017) was carried out at the individual marker and sample levels. Marker filtering included an assessment of 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 of individuals that don't have a genotype at a locus, the quality of the sequencing run, the number of SNPs at a locus (considers whether there are SNPs from different parts of the chromosome – i.e. paralogous loci - with 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.

If an individual is missing data above a threshold because of poor-quality DNA, they are removed.

2. Genome-wide average heterozygosity.

The position at which a SNP occurs on a chromosome is called the locus (plural loci). Because SNPs are bi-allelic, they contain two alleles at each locus. Heterozygosity is a measure of how many loci contain two different alleles (heterozygous genotype) versus how many loci have two identical alleles (homozygous genotype). On average individuals within a population will have the same level of heterozygosity as each other. However, if the heterozygosity observed for the DNA profile of an individual deviates from this average then this likely reflects sample cross contamination – introduced 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). Conversely, samples with lower than average heterozygosity are likely an indication of poor DNA quality that results in a homozygous excess because of introduced artefactual sequencing bias. An important step in assessing the quality of samples is therefore to identify samples that are either too homozygous or too heterozygous compared to the average observed level of heterozygosity. To do this, the level of genome-wide mean heterozygosity is calculated. For the current study, individual

samples with a mean heterozygosity above and below statistical threshold values of higher and lower confidence limits and are filtered out of datasets for further quality control. For the current study, samples with a mean heterozygosity that did not deviate from the mean by more than 25% were retained in the dataset.

3. Highly similar/duplicate genotypes.

Genetic similarity is used to identify individuals that are closely related where more closely related individuals show higher levels of genetic similarity and by extension, show lower levels of genetic distance between them relative to average genetic distance between unrelated pairs. In essence, non-related individuals should have genotypes that are dissimilar (because they have no common relatives to derive their genes from). However, when cross-contamination or technical mishaps occur (e.g. labelling two samples collected from the same individual as different animals), samples with similar or almost identical genotypes can occur among individuals sampled from a population. Care needs to be taken in examining individuals with similar genotypes to determine if values of genetic distance are reflective of relatedness or the result of human error. In addition, the sequencing process includes a number of technical replicates. These are included to examine the repeatability of sequencing results and so therefore need to be removed prior to any further analysis of sequencing results.

Samples were further filtered prior to modelling by removing those markers with less than 5% presence in at least one region/year. This avoided markers that were very rare and hence not informative. Samples that were of a genetic distance (based on the Manhattan distance) of less than 0.01% of the rest of the pairs in the data set and considered to be abnormally close, were also removed.

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 genetically distinct groups 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 genetically distinct 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 genetically distinct group (Foster et al. 2018). This mixture model allows for the identification of contemporary differences in genetic groupings as opposed to admixture models (e.g. ADMIXTURE, STRUCTURE) which focus on the identification of historical groupings, noting that in some instances contemporary and historical groupings will be the same. Further, because this mixture model allows for the identification of contemporary differences in genetic groupings, it obviates the need to separate neutral and putative loci. See Foster et al. (2018) for a full discussion of the limitations and misuse of many of the multi-model approaches commonly used by researchers investigating the population structure of animal populations. Further, when compared to admixture models Foster et al. (2018) clearly demonstrated that the mixture model StockR performs better than all other programs available.

Two approaches are utilised in StockR 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 groups 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 genetically distinct 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 the analysis, and are only applied for 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

Sample Collection

The spatial distribution of samples and numbers of samples included in the project is provided in Table 1 and Figure 1.

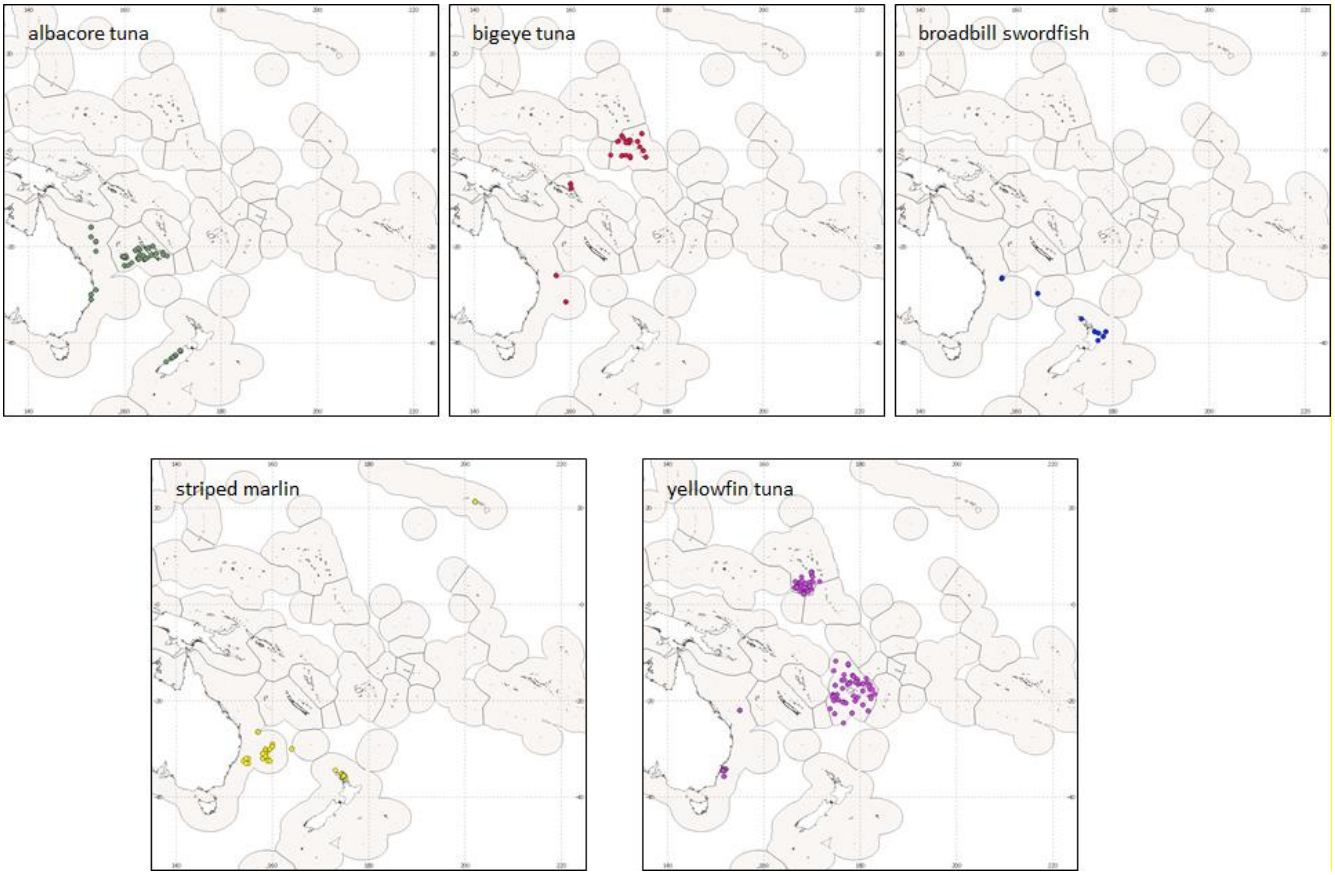

Figure 1. The spatial distribution of samples collected from albacore, bigeye and yellowfin tunas, broadbill swordfish and striped marlin. Each dot represents either the location of the fishing event in which the sample was collected (where the exact fishing location was available) or the centre of the area fished (represented by boundary coordinates provided by the fishing company) in which multiple samples were collected (where samples were collected at the processing factory on land and could not be attributed to individual fishing events).

Accessing samples from broadbill swordfish from two sites within the WCPFC area was particularly problematic with several potential sampling avenues proving unfruitful. Although a New Zealand processor was identified by late 2018, a poor fishing season in 2019 resulted in no samples being collected from New Zealand in 2019 and any sampling being delayed to 2020. This poor fishing season also impacted sampling from striped marlin from New Zealand, resulting in only 15 samples able to be collected. A change in processor in New Zealand and agreement by the Cook Islands Ministry of Marine Resources to collect samples from broadbill swordfish has now resulted in samples being collected from the two locations in 2020. Lockdowns associated with the COVID-19

pandemic in New Zealand delayed sample collection as export markets closed and domestic markets shrank, resulting in the accrual of 50 samples from broadbill swordfish taking longer than expected. The COVID-19 pandemic also shut down all broadbill swordfish fishing operations in the Cook Islands with sampling yet to resume at the time of submission of this report. 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 (see also discussion of next steps in the Conclusion).

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

Quality of samples

Examination of gel runs on DNA extracted from yellowfin tuna samples initially received from the WCPFC Tuna Tissue Bank (Fiji and Marshall Islands) identified 49 samples that had degraded to the point that very little high molecular weight DNA could be extracted, which is necessary for the DArTseq™ technique (Table 1). The catch location of a further one sample was unable to be confirmed by Pacific Community staff. As a result, 50 samples did not progress to genetic sequencing (Table 1). Replacement of poor quality samples by other samples in the WCPFC Tuna Tissue Bank was required and facilitated in early 2018. Examination of gel runs from an initial 148 striped marlin samples identified 35 that had degraded to the point that insufficient amounts of DNA were able to be extracted and these could not be sequenced. DNA extracts of sufficient quality were obtained from all other samples and these were progressed to sequencing.

Species identification

Species identification revealed a total of eleven samples where species had been misidentified or mislabelled (Table 1). Four samples from the Marshall Islands originally identified as bigeye tuna were yellowfin tuna, one sample from the ETBF originally identified as broadbill swordfish was likely a striped marlin, two samples from Hawai'i originally identified as striped marlin were likely to be short-billed sailfish and four samples from the Marshall Islands originally identified as yellowfin tuna were bigeye tuna. All misidentified/mislabelled samples were removed from further analysis.

Quality control of sequencing data

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

Missing data

When a threshold of permissible missing data was set at 15%, a total of 32 albacore tuna and nine yellowfin tuna samples were removed from subsequent population analysis (Table 1).

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

Species	Region/EEZ	Year	No. samples collected/received	No. samples removed due to poor DNA quality	No. genotypes produced (incl. technical replicates)	No samples removed due to quality control steps				Final no. samples included in analyses
						Incorrect species ID	Missing data	Heterozygosity	Similar genotypes	
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

Species	Region/EEZ	Year	No. samples collected/received	No. samples removed due to poor DNA quality	No. genotypes produced (incl. technical replicates)	No samples removed due to quality control steps				Final no. samples included in analyses
						Incorrect species ID	Missing data	Heterozygosity	Similar genotypes	
		2015	50	—	50	1	—	3	—	46
Bigeye	Solomon Islands	2013	56	—	56	—	—	2	7 (all technical replicates)	47
		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

Species	Region/EEZ	Year	No. samples collected/received	No. samples removed due to poor DNA quality	No. genotypes produced (incl. technical replicates)	No samples removed due to quality control steps				Final no. samples included in analyses
						Incorrect species ID	Missing data	Heterozygosity	Similar genotypes	
		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
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

Species	Region/EEZ	Year	No. samples collected/received	No. samples removed due to poor DNA quality	No. genotypes produced (incl. technical replicates)	No samples removed due to quality control steps				Final no. samples included in analyses
						Incorrect species ID	Missing data	Heterozygosity	Similar genotypes	
		2015	52	2	58	3	—		9 (all technical replicates)	46

Genome-wide average heterozygosity

Genome-wide average heterozygosity values were highest in albacore samples from New Caledonia with the highest number of samples with average heterozygosity values above the overall mean also derived from New Caledonia (Figure 2). Samples from New Zealand had higher average heterozygosity value than samples from the ETBF, which had the lowest average heterozygosity values of the three locations (Figure 2). Thirty one samples from New Caledonia, ten samples from New Zealand and one sample from the ETBF were above the confidence limit threshold and subsequently removed from further analysis (Table 1).

Across bigeye tuna samples genome-wide average heterozygosity was highest in samples from the ETBF, with the highest number of samples with average heterozygosity values above the overall mean also occurring in samples from the ETBF (Figure 2). The overall average heterozygosity value of samples from the Marshall Islands was slightly higher than that of samples from the Solomon Islands, which had the lowest average heterozygosity values of the three locations (Figure 2). A total of 85 samples from the ETBF, nine samples from the Marshall Islands and three samples from the Solomon Islands were above the confidence limit threshold and were removed from further analysis (Table 1).

Genome-wide average heterozygosity values were highest in broadbill swordfish samples from the ETBF, with the highest number of samples with average heterozygosity values above the overall mean occurring in samples from New Zealand. The overall average heterozygosity of samples from New Zealand was slightly higher than the overall average heterozygosity of samples from the ETBF and Norfolk Island, although samples from all locations appeared to have similar average heterozygosity values (Figure 3). One sample from each of the ETBF and New Zealand were above the confidence limit threshold and were removed from further analysis (Table 1).

Amongst striped marlin samples, genome-wide average heterozygosity was highest in samples from Hawai'i, with the highest number of samples occurring above the overall mean also derived from Hawai'i (Figure 3). The overall average heterozygosity of samples from New Zealand was slightly higher than the overall average heterozygosity of samples from the ETBF, although samples from both locations appeared to have similar average heterozygosity values (Figure 3). A total of 59 samples from Hawai'I, 21 samples from New Zealand and 16 samples from the ETBF were above the confidence limit threshold and removed from further analysis (Table 1).

Genome-wide average heterozygosity values for yellowfin tuna were highest in samples from the ETBF, with the highest number of samples with average heterozygosity values above the overall mean occurring in samples from Fiji (Figure 3). The larger number of samples with values above the overall mean resulted in the overall average heterozygosity of samples from Fiji being higher than the other two locations. The overall average heterozygosity of samples from the ETBF was slightly higher than the overall average heterozygosity of samples from Marshall Islands, which had the lowest average heterozygosity values of the three locations (Figure 3). A total of 45 samples from Fiji, 15 samples from the ETBF and three samples from the Marshall Islands were above the confidence limit threshold and removed from further analysis (Table 1).

Highly similar/duplicate genotypes

The majority of sequenced individuals identified as having similar genotypes were either DArT technical replicates (repeated DNA libraries used as statistical replicate samples). The remainder were replicate tissue samples included to evaluate potential differences caused by the two different extraction methods. This included 38 albacore tuna samples 30 bigeye tuna samples, five broadbill swordfish samples, 16 striped marlin samples and 3 yellowfin tuna samples. The relative genetic distances of all samples were also checked. Alack of samples showing relative genetic distances less

than 75% indicates there were no observed fish related to each other at either full-sib or half-sib levels that could potentially violate assumptions of the population genetic analysis.

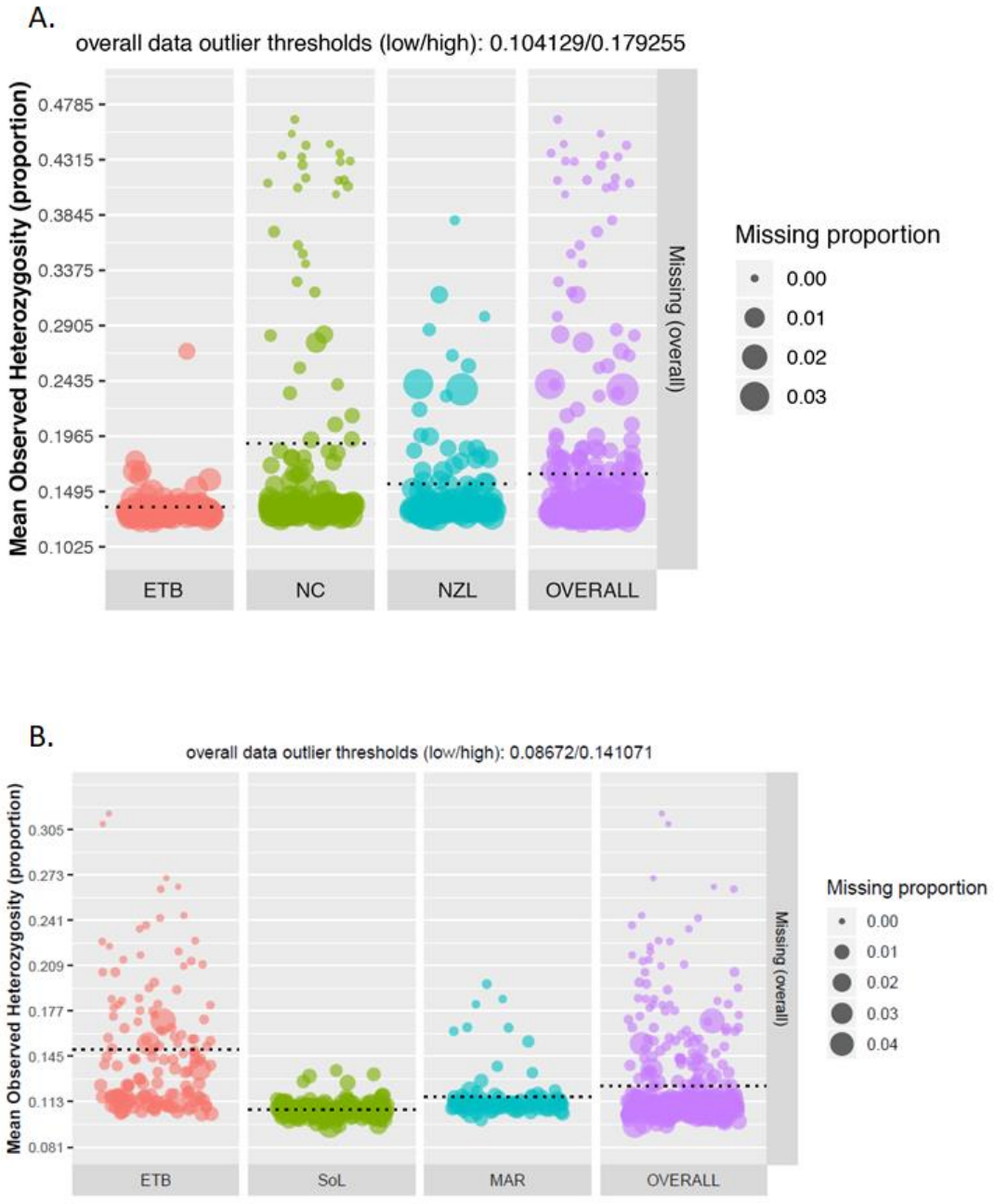

Figure 2. Genome-wide mean observed heterozygosity for individual (A) albacore tuna from the ETBF, New Caledonia (NC) and New Zealand (NZ) and (B) bigeye tuna from the ETBF (ETB), Solomon Islands (SoL) and Marshall Islands (MAR). Dashed horizontal lines indicate the average heterozygosity for each dataset. Statistical threshold values of higher and lower confidence limits above and below which samples were removed were 0.104129 (low) and 0.179255 (high) for albacore tuna and 0.08672 (low) and 0.141071 (high) for bigeye tuna. Size of data points represents the proportion of missing data in each sample.

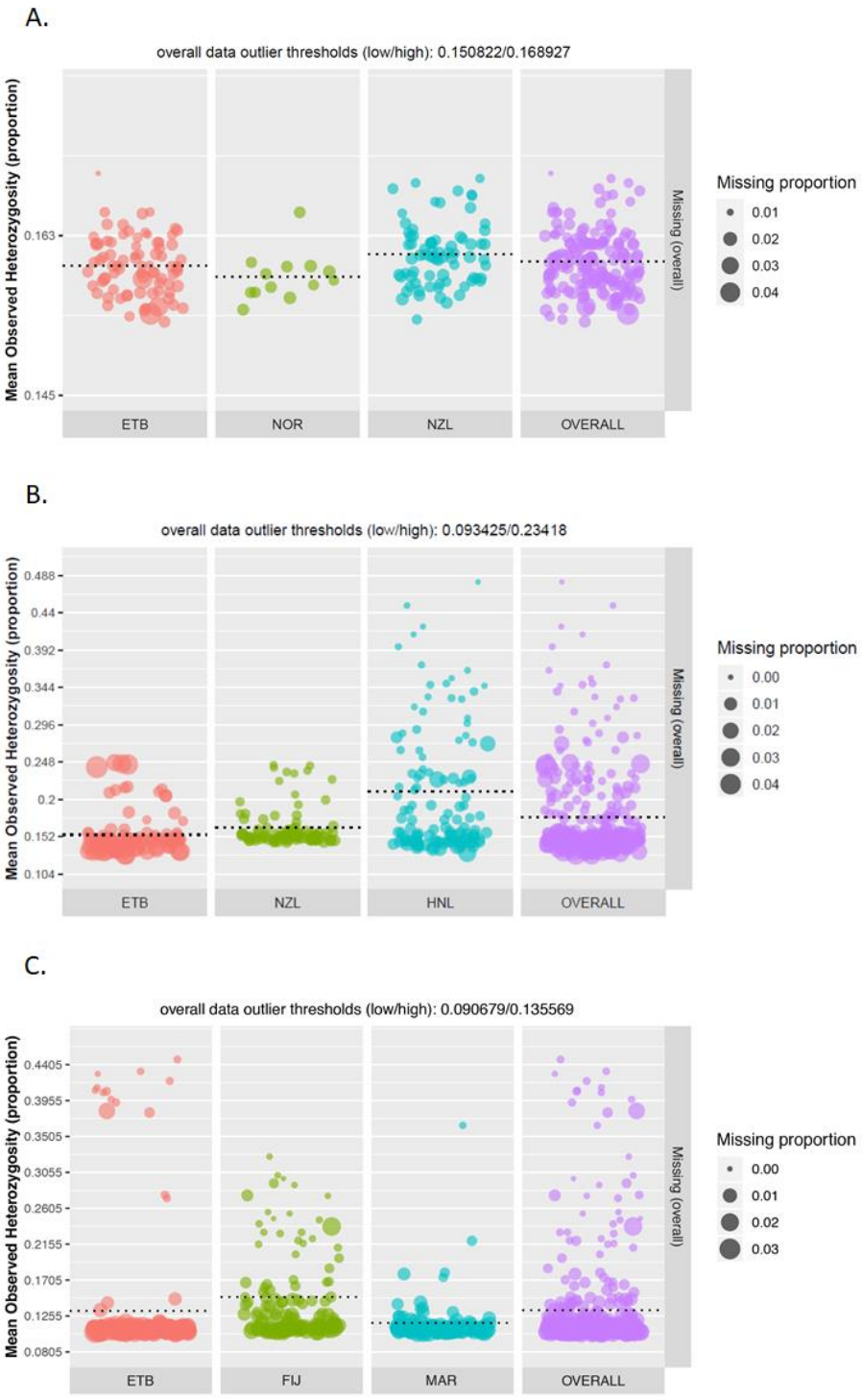

Figure 3. Genome-wide mean observed heterozygosity for individual (A) broadbill swordfish from the ETBF (ETB), Norfolk Island (NOR) and New Zealand (NZL) (B) striped marlin from the ETBF (ETB), New Zealand (NZL) and Hawai'i (HNL) and (C) yellowfin tuna from the ETBF (YFT-ETB), Fiji (YFT-FIJ) and the Marshall Islands (YFT-MAR). Dashed horizontal lines indicate the average heterozygosity for each dataset. Statistical threshold values of higher and lower confidence limits above and below which samples were removed were 0.150822 (low) and 0.168927 (high) for broadbill swordfish, 0.093425 (low) and 0.23418 (high) for striped marlin and 0.090679 (low) and 0.135569 (high) for yellowfin tuna. Size of data points represents the proportion of missing data in each sample.

Population modelling

Albacore tuna

A total of 14,298 SNPs from albacore tuna were included in the mixture model. On the basis of AIC and BIC calculated by the model, the number of groups (K) that minimised the information criteria was identified as one (Figure 4). Cross validation assigned 100 percent of all markers to K=1. This dropped to 79.2 percent at K=2 and 51.6 percent at K=3. The probability of assignment was consistent amongst years at all three sampling locations (ETBF, New Caledonia, New Zealand). The results from the mixture model and cross validation at K=2 suggest the possibility of a second group that is more prevalent in samples from New Zealand, however assignment was statistically highly uncertain and resulted in some individuals not being able to be assigned to either group in the scenario with any confidence (Figure 4). As such, the results suggest very little genetic differentiation among the three sampling locations, which is consistent with our ability to resolve a single genetic grouping of albacore tuna with regard to the three sites and the years examined.

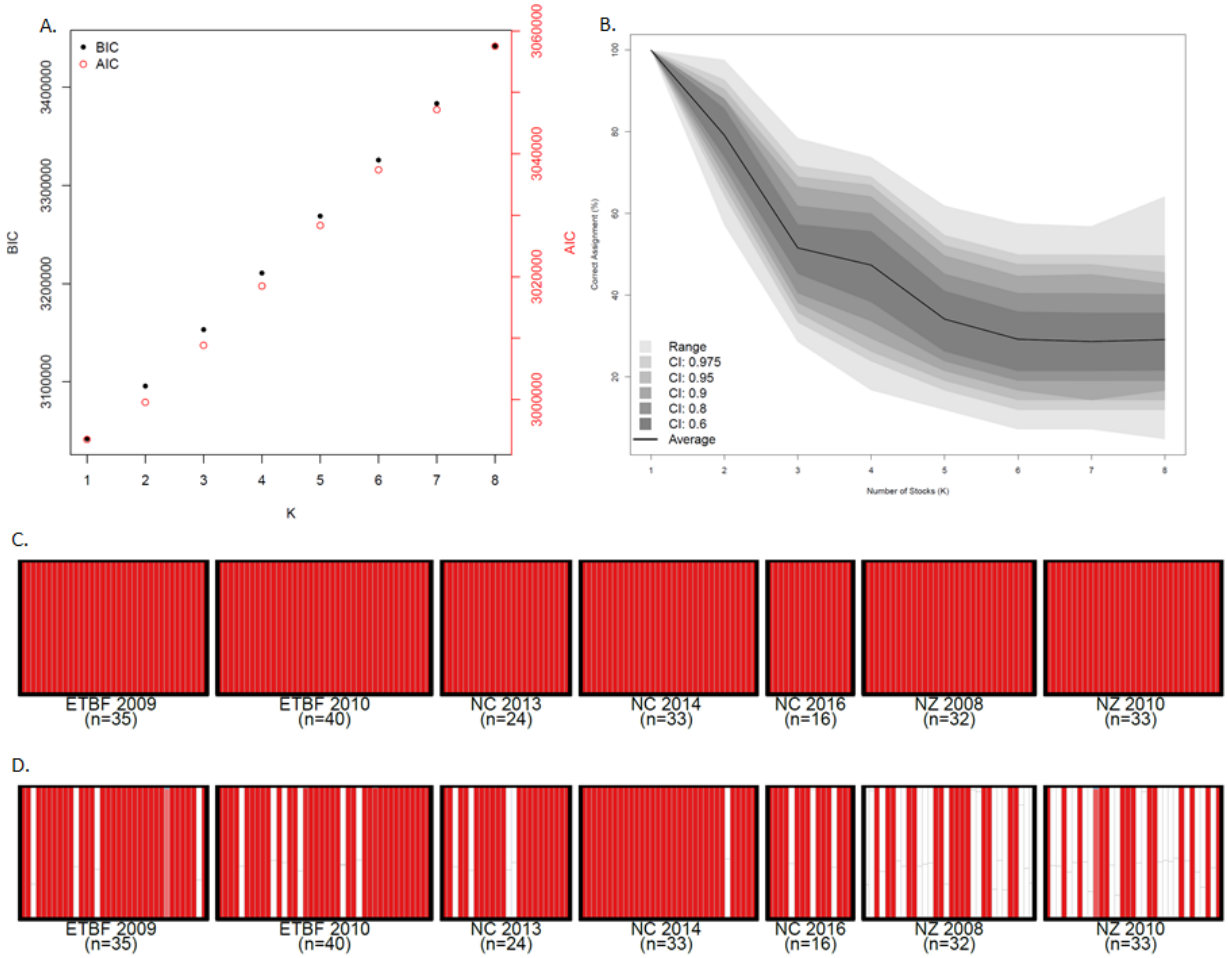

Figure 4. Output of the mixture model for albacore tuna. A. Information criterion given K=1…8. Note the scales for each information criterion varies. B. Cross validation and percent assignment of markers assuming K=1-8. The probability of individual assignment to C. K=1 and D. K=2. Weakening (whiter) of colours 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. ETBF: ETBF, NC: New Caledonia, NZ: New Zealand.

Bigeye tuna

A total of 5,695 SNPs from bigeye tuna were included in the mixture model. On the basis of AIC and BIC calculated by the model, the number of genetic groups (K) that minimised the information criteria was one (Figure 5). Cross validation assigned 100 percent of all samples to K=1. This dropped to 78.4 percent at K=2 and 58.6 percent at K=3. The probability of assignment was consistent amongst years at all three sampling locations (ETBF, Marshall Islands, Solomon Islands). Given the information criteria and cross validation results, the data do not support the discrimination of more than one genetic group of bigeye tuna across the three sampling locations and amongst the years examined.

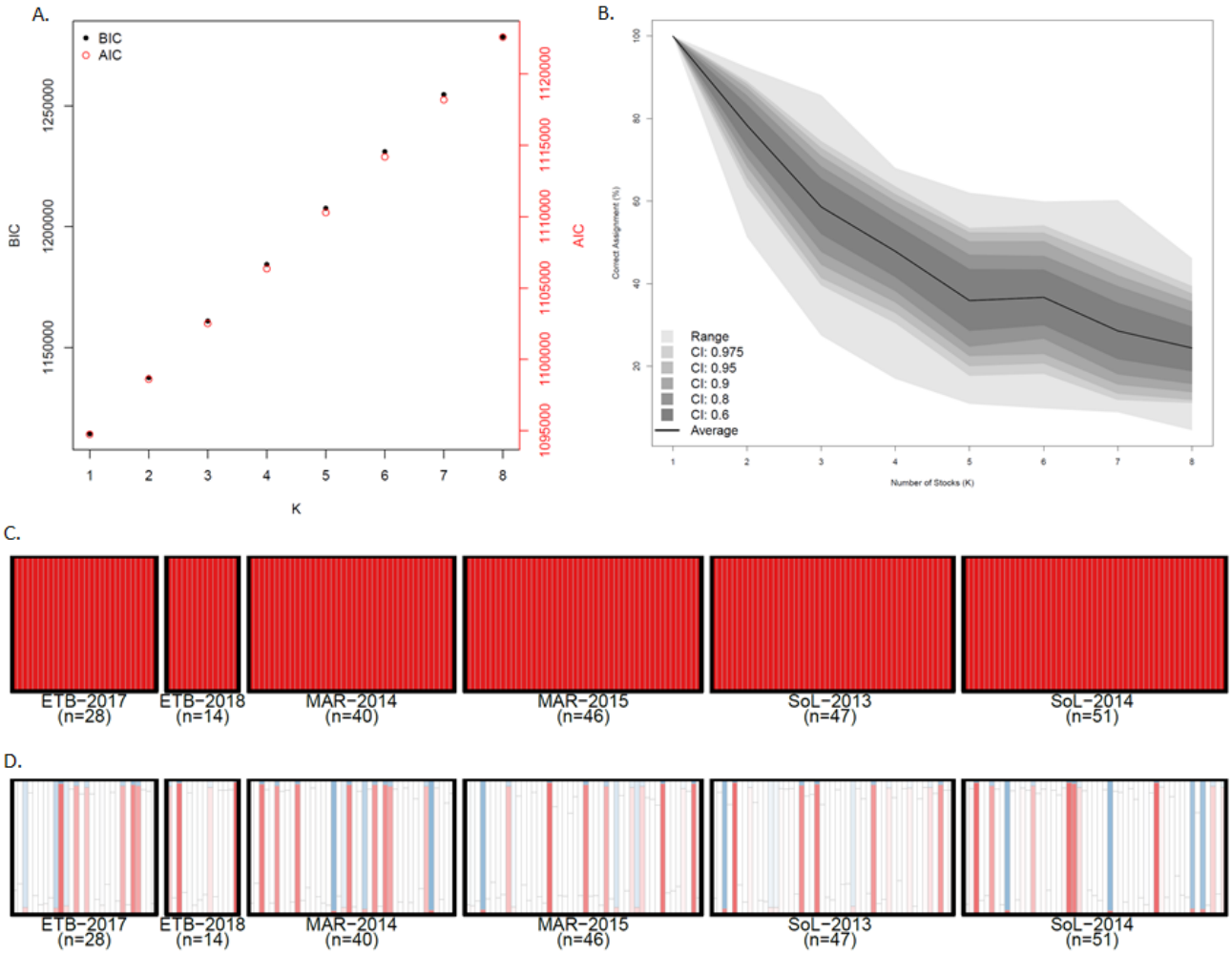

Figure 5. Output of the mixture model for bigeye tuna. A. Information criterion given K=1 to K=8. Note the scales for each information criterion varies. B. Cross validation and percent assignment of markers on the basis of K=1 to K=8. The probability of individual assignment to C. K=1 and D. K=2. Weakening 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. ETB: ETBF, MAR: Marshall Islands, SoL: Solomon Islands.

Broadbill swordfish

A total of 14,442 SNPs from broadbill swordfish were included in the mixture model. On the basis of AIC and BIC calculated by the model, the number of genetic groups (K) that minimised the information criteria was one (Figure 6). Cross validation assigned 100 percent of all samples to K=1. This dropped to 66.9 percent at K=2 and 49.3 percent at K=3. The probability of assignment was consistent amongst years in samples from the ETBF. Given the information criteria and cross validation results, the data do not support the discrimination of more than one genetic group of broadbill swordfish across the three sampling locations (ETBF, New Norfolk, New Zealand) and amongst the years examined.

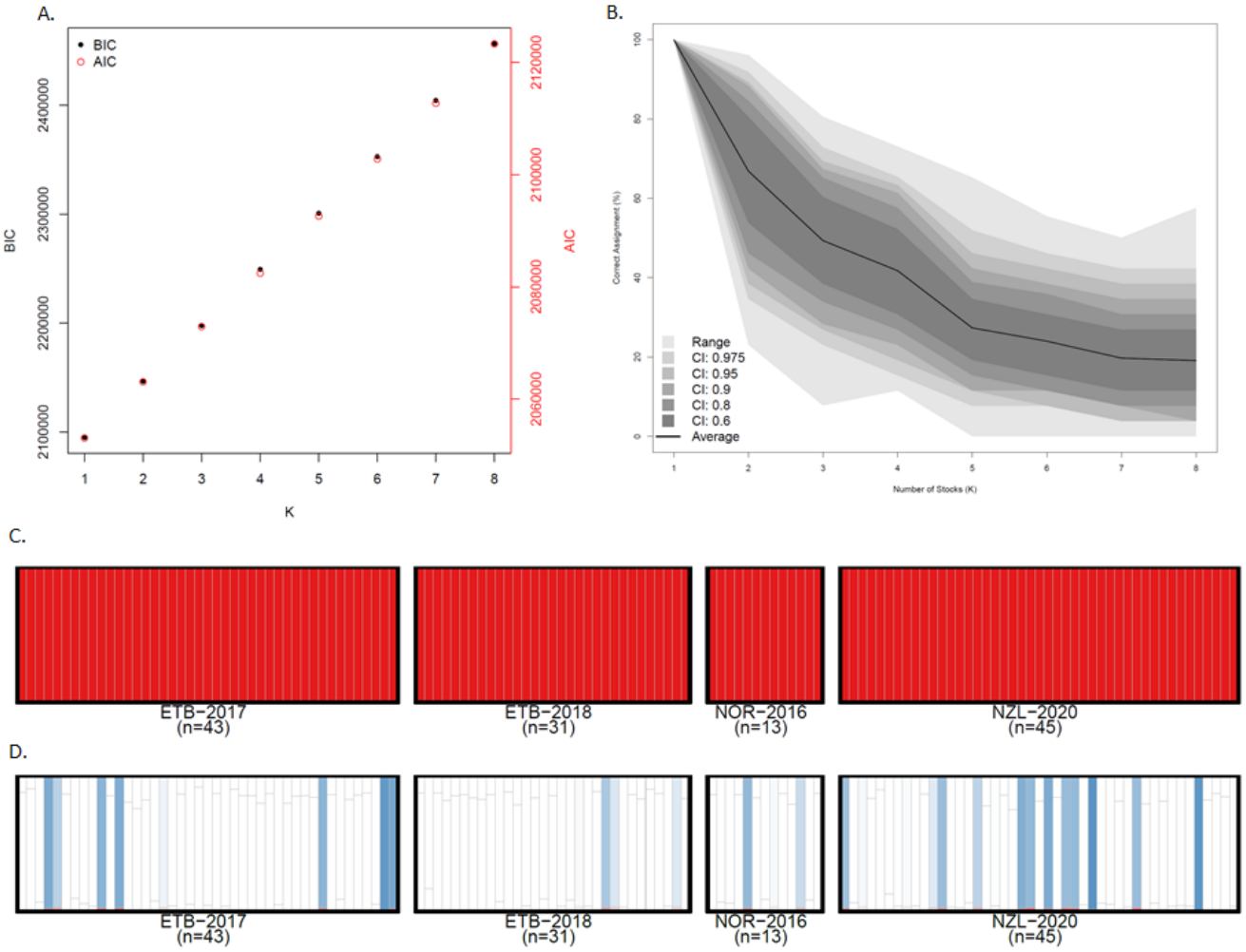

Figure 6. Output of the mixture model for broadbill swordfish. A. Information criterion given K=1 to K=8. Note the scales for each information criterion varies. B. Cross validation and percent assignment of markers on the basis of K=1 to K=8. The probability of individual assignment to C. K=1 and D. 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. ETB: ETBF, NOR: Norfolk Island, NZL: New Zealand.

Striped marlin

A total of 11,311 SNPs from striped marlin were included in the mixture model. On the basis of AIC and BIC calculated by the model, the number of genetic groups (K) that minimised the information criteria was two (Figure 7). Cross validation assigned 100 percent of all samples to K=1 and 99.7 percent for K=2. This dropped to 79.1 percent at K=3 and 57.5 percent at K=4. The results indicate that there may be two genetic groups of striped marlin within these data, with the ETBF, New Zealand and Hawai'i sharing the first group. The second (less sampled group) was identified only from samples collected from Hawai'i. The probability of assignment was consistent amongst years in samples from the ETBF and New Zealand. Sampling of only one year from Hawai'i precluded any assessment of temporal stability in assignment to genetic groups from this site.

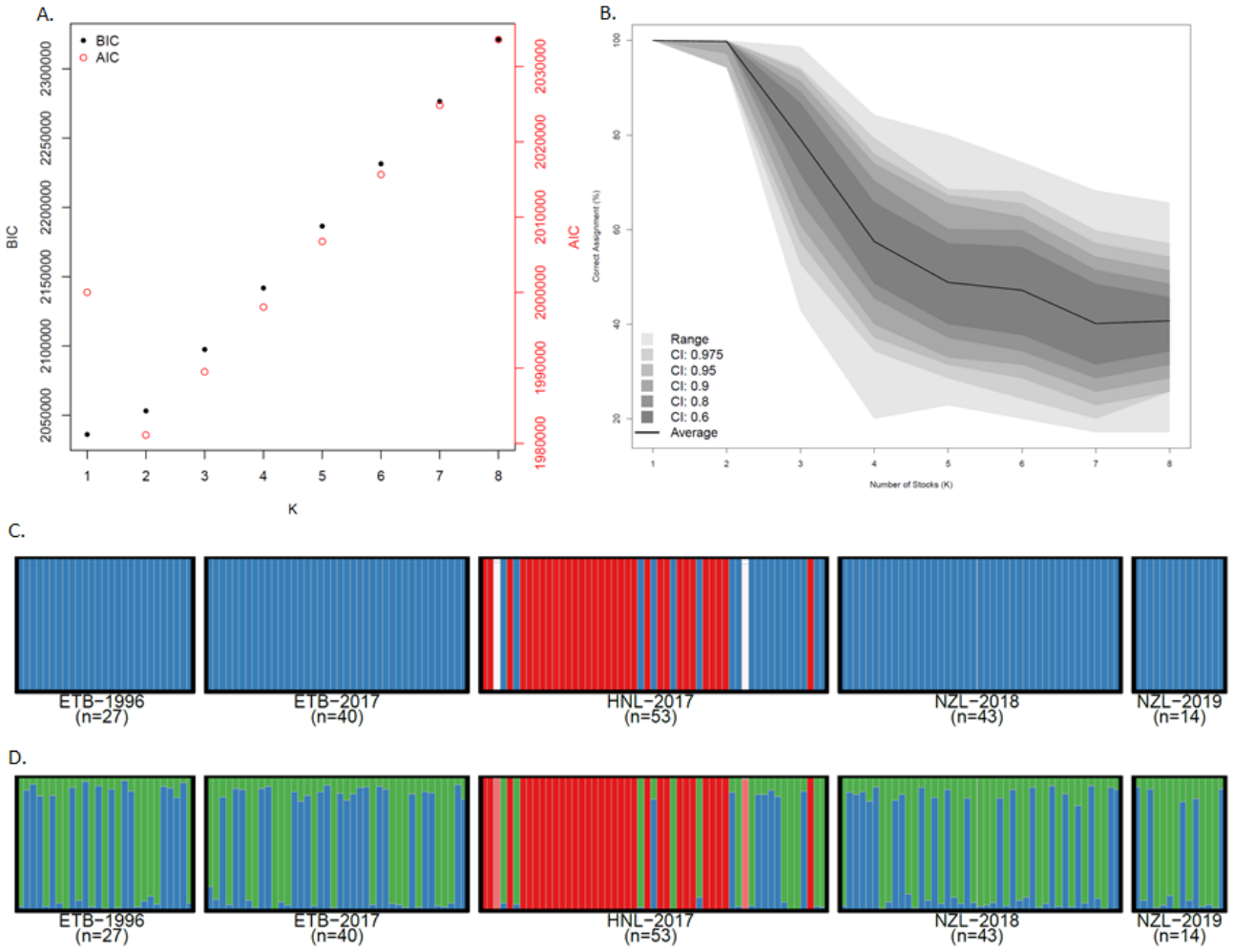

Figure 7. Output of the mixture model for striped marlin. A. Information criterion given K=1 to K=8. Note the scales for each information criterion varies. B. Cross validation and percent assignment of markers on the basis of K=1 to K=8. The probability of individual assignment to C. K=2 and D. K=3. 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. ETB: ETBF, HNL: Hawai'i, NZL: New Zealand.

Yellowfin tuna

A total of 9,731 SNPs from yellowfin tuna were included in the mixture model. On the basis of AIC and BIC calculated by the model, the number of genetic groups (K) that minimised the information criteria was identified as one (Figure 8). Cross validation assigned 100 percent of all markers to K=1. This dropped to 73.8 percent at K=2 and 61.6 percent at K=3. The probability of assignment was consistent amongst years at all sampling locations (ETBF, Fiji, Marshall Islands). Given the results from the mixture model and cross validation, the data do not support the discrimination of more than one genetic group of yellowfin tuna across the three sites and the years examined.

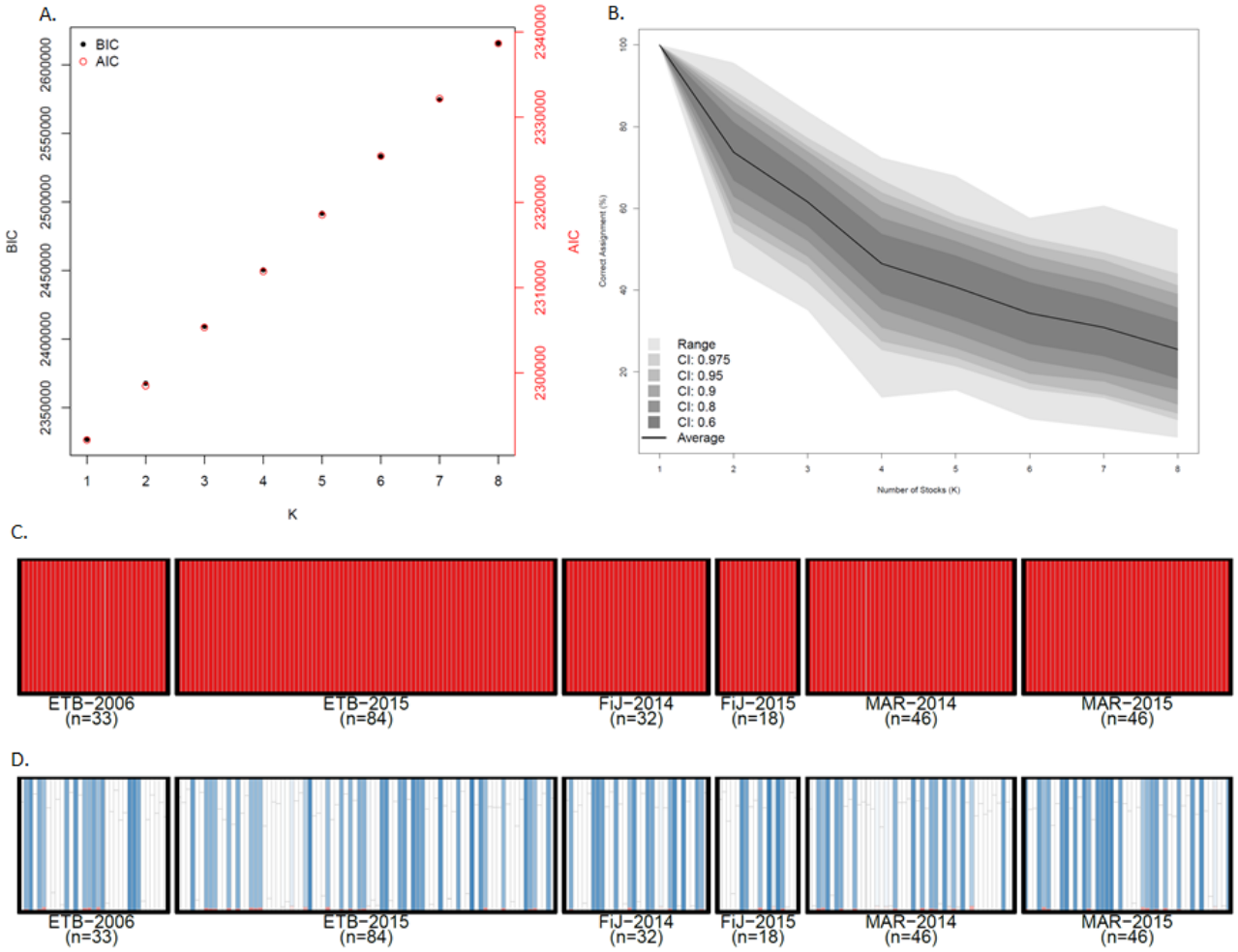

Figure 8. Output of the mixture model for yellowfin tuna. A. Information criterion given K=1 to K=8. Note the scales for each information criterion varies. B. Cross validation and percent assignment of markers on the basis of K=1 to K=8. The probability of individual assignment to C. K=1 and D. K=2. 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. ETB: ETBF, FIJ: Fiji, MAR: Marshall Islands.

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 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. Consistency in results across years suggest that the groupings revealed here have some temporal stability across years across most sites (note samples collected from Hawai'I, Norfolk Island and New Zealand constitute one year only). Although results suggest the potential for a second genetic grouping among albacore samples, assignment by the methods used here was statistically uncertain and resulted in some individuals not being able to be assigned to either group in the scenario with any confidence. Further sampling from the three locations included here as well as inclusion of samples from additional sites (with temporal replication) would also be required for resolving these uncertainties.

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 with any certainty. 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 across studies. Further sampling and analysis of samples from additional sites across the western and central Pacific using the same methods used in this study 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). More recent reanalyses of those samples reported in Grewe et al. (2015), in the context of increased sample locations across the Pacific, Indian and Atlantic Oceans, utilising the same data quality control pipelines and modelling approaches as those used in this study, have identified that the differentiation between samples from Tokelau and the Coral Sea is not as substantive as first reported (Grewe et al. unpublished data). This highlights the need for care when comparing studies utilising differing analytical approaches and recognition that variation in approaches can limit comparisons.

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).

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. 2020b).

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 swordfish caught at different locations. 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, samples collected across 2020 and 2021 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. These results will be presented to the WCPFC Scientific Committee in 2021.

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 south-western Pacific Ocean.

The spatial dynamics of the population components of tuna and billfish that might contribute to models of genetic isolation by distance (dispersion of larvae, distribution of spawning adults) across the western and central Pacific Ocean are either largely unknown or have been inferred through the analyses of catch data. However, what is known of the distribution of the larvae of the five species included in this study suggest that there is some continuity in their distribution longitudinally at least in the tropical waters of the western Pacific Ocean and in waters of the ETBF and those that are adjacent to the ETBF. Scombrid larvae, indicative of active spawning of tuna have been observed in plankton tows in the northern waters of the Great Barrier Reef (Leis and Goldman 1984). Although not identified to species by Leis and Goldman (1984), larval tows conducted by others in this region have identified larvae of albacore, bigeye and yellowfin tunas with the highest densities observed between October and December (Nishikawa et al. 1985). Across the same months, high densities of albacore tuna larvae have been observed between 10-20˚S and 160-180˚E and broadbill swordfish larvae have been observed across a similar latitudinal range but between 150-160˚E (Nishikawa et al. 1985). High densities of bigeye tuna larvae have also been observed east of Paua New Guinea during the months of October through to December (Nishikawa et al. 1985). Low numbers of striped marlin larvae have been observed in the waters of Hawai'i during May (Hyde et al. 2006), with higher densities observed west of 130 ˚W between 10-30˚S during November and December (Nakamura 1983). Yellowfin tuna larvae have been caught east of Papua New Guinea during the period October to December (Nishikawa et al. 1985) and in the waters of French Polynesia during January to March (Strasburg 1960). The duration of pelagic phases of larval life stages are known to influence population- level patterns of genetic connectivity (Selkoe and Toonen 2011), however there are few data on either the duration or dispersal of larval stages tuna and billfish species across the western and central Pacific Ocean.

Aggregations of mature-sized bigeye and yellowfin tunas in the northern Coral Sea have been associated with spawning (McPherson 1988). Examination of the gonads of female yellowfin tuna from the northern Coral Sea region of the ETBF documented advanced stages of oocyte maturation, suggesting individuals were spawning in the region during the months of October and November (McPherson 1991). Examination of the gonads of striped marlin suggest spawning occurs in northern parts of the ETBF predominantly during the austral summer months (Kopf et al. 2012). Broadbill swordfish in the east Australian region, at latitudes lower than 25°S demonstrate a protracted spawning season with spawning occurring in waters with surface temperatures of greater than 24°C between September and March and peaking in December to March (Young et al. 2003).

How spatially dispersed spawning of bigeye and yellowfin tunas might occur across the western and central Pacific Ocean, particularly in equatorial regions, is largely unknown and if spawning does occur in distinct locations, the degree to which individuals demonstrate fidelity to those locations is also unknown (Moore et al. 2020b). However, spawning of albacore tuna is known to occur during the austral spring and summer months in a region that extends from the Coral Sea to waters around French Polynesia (Farley et al. 2013). Spawning populations of bigeye tuna have been estimated to occur in equatorial regions from Papua New Guinea across to French Polynesia (Lehodey et al. 2008). Spawning populations of yellowfin tuna also occur across equatorial regions, however, have a more restricted longitudinal range, largely occurring in association with the western warm pool which occurs predominantly west of 180˚E, although moves longitudinally with the El Niño Southern Oscillation (see Picaut et al. 1996; Lehodey et al. 2003). This suggests the potential that spawning individuals in close proximity to each other may mix to a higher degree than those that are more spatially separated, again supporting proposed models for albacore, bigeye and yellowfin tunas of isolation by distance. The results of this study suggest that in waters west of around 170 ˚E, the three tuna species, as well as broadbill swordfish, may mix to a degree that has resulted in the limited genetic differentiation between the locations included here.

Relevance for the implementation of harvest strategies in the ETBF

Harvest strategies developed for bigeye tuna and yellowfin tuna have been based on stock assessments carried out under the WCPFC and those currently being developed for striped marlin similarly are based on stock assessments carried out under WCPFC (Hillary et al. 2016; Rich Hillary pers comm.). These assessments assume broad scale mixing of individuals across the WCPFC area, although assessments include some disaggregation of data into regions to describe some spatial processes such as recruitment and movement (McKechnie et al. 2017; Tremblay-Boyer et al. 2017; Ducharme-Barth et al. 2019). The operating models currently being developed for broadbill swordfish as part of revisions to the harvest strategy is being run under two likely scenarios, one where there is mixing of broadbill swordfish between the ETBF and the western Pacific Ocean and one where these is no mixing between the ETBF and the western Pacific Ocean (Rich Hillary, pers comm). Given the operating models being used and scenarios for testing of harvest strategies, the results presented here are consistent with outcomes already being considered under these processes and are unlikely to change outcomes. 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. Results from the project lend support to current assessment structures undertaken across the WCPFC Area and provide confidence in ensuring that management measures set under domestic structures align with regional management measures.

Considerations on sample quality

Degradation of DNA in tissue samples can occur for a number of reasons including 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 during transit from vessels or the market to archives (e.g. thawing of samples during transit), or 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.

Considerations on sampling design

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 discern 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 would 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.

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 (and substantive support) to achieve (see Moore et al. 2020b).

Considerations for current sample archives

Without access to muscle tissues archived in the WCPFC Tropical Tuna Tissue Bank, this project would not have been possible. Although the WCPFC Tropical Tuna Tissue Bank was not initially set up to support genetic analyses, it is increasingly being used as a resource for accessing samples for genetic studies (e.g. this study, Anderson et al. 2019a, 2019b). 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 further developed to better support population structure studies (see also Appendix 2). These include:

I. Species coverage

Tissues currently contained in the WCPFC Tropical 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. shark species). 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 and population connectivity.

II. Spatial coverage

Tissues currently contained in the WCPFC Tropical Tuna Tissue Bank to some extent reflect the distribution of the highest catches across the WCPFC area. As a result, there are particular regions where samples are almost 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 and population connectivity.

III. Sample sizes

Tissues currently contained in the WCPFC Tropical Tuna Tissue Bank, whilst impressive overall, rapidly decline in numbers once distributed on the basis of species, sample type and spatial and temporal qualifiers. In particular, the utility of the Tissue Bank declines for stock structure and population connectivity 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 and then across a number of years. This declines further when attempting to target samples from particular year classes or cohorts and align samples with gonad samples for the identification of spawning individuals. Greater focus on building tissue samples from a small number of regions across multiple years (these regions could vary through time) and from particular age classes and/or reproductive state would substantially increase the utility of the Tissue Bank for stock structure and population connectivity studies.

IV. Quality of samples

The quality of samples included in this study varied considerably with misidentification/mislabelling of species, samples of low DNA quality and cross-contamination identified across datasets. Issues with species identification of billfish have been identified from the WCPFC observer program in the past (Williams et al. 2018). The incidence of these factors has flow on effects on overall sample numbers for analysis and highlights the need for first, strict sample collection and handling protocols including species identification tools and capability and second, appropriate data quality control processes to be factored into studies. Without strict quality control measures, particularly those that allow for the identification of cross contamination, the potential for misinterpretation of data is increased. In this study sample sizes were reduced by as much as 53 individuals in any one year

(yellowfin tuna from Fiji). Such large reductions in sample sizes reduce the robustness of any analyses, reducing confidence in results and have flow on impacts on the applicability of sample archives such as the WCPFC Tropical Tuna Tissue Bank for stock structure and population connectivity studies.

Conclusion

This project aimed to investigate the presence of stock structure in the five principal species caught in Australia's Eastern Tuna and Billfish Fishery and the western Pacific Ocean across spatial scales of relevance using current, state of the art genomic methods. Achievement of these goals was largely successful despite challenges in accessing samples and variable sample quality across regions. Results suggest that albacore, bigeye and yellowfin tuna as well as broadbill swordfish caught in the ETBF cannot be genetically discriminated from fish collected from locations in nearby regions in the western Pacific and that there is some genetic connectivity of striped marlin caught in the ETBF as far east as Hawai'i.

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. However, a caveat to these results is that they *only apply to the sites* included for each of the species examined in this study and therefore *cannot* be extrapolated across the wider western and central Pacific Ocean region with any certainty. Further sampling and analysis of samples from additional sites across the western and central Pacific, including temporal replication at those sites, are required to investigate whether the results presented here represent a consistent pattern across the western and central Pacific region or whether greater genetic differentiation is discernibly present. The resources required to support the attainment of broader insights into the connectivity of species across the WCPFC area and connectivity between the ETBF and the western and Central Pacific Ocean will be dependent on current access to samples, the extent of further sampling required in order to attain broad spatial and temporal coverage of samples, the facilities and capability available for processing and sequencing samples and the capability available for data quality control and analysis pipelines. It is likely that the efforts required to establish such broadscale insights will not be insignificant, just given the spatial extent of the WCPFC area.

As next steps, a second year of sampling for broadbill swordfish from New Zealand is planned and a preliminary small dataset from the Cook Islands (consisting of 24 samples) has been collected. These samples will be analysed and incorporated with the data from this project to provide further insights into the connectivity of broadbill swordfish across the western and central Pacific Ocean and presented to the WCPFC Scientific Committee in August 2021.

This project and others investigating the stock structure of tuna species in the Indo-Pacific region (see Proctor et al. 2019; Grewe et al. 2019; Davies et al. 2020) have generated wide interest in reducing uncertainties in the stock structure of these species within the context of current development of harvest strategies and management frameworks under the WCPFC. In 2018, a regional workshop was held to identify current understanding of the spatial dynamics and connectivity of albacore, bigeye, skipjack and yellowfin tunas and what would need to be undertaken as a body of work in order to resolve the stock structure of the four species (Moore et al. 2020a; 2020b). In order to determine if multiple spawning populations for 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. 2020b). As part of the discussions generated through regular reporting of this project to the WCPFC, the WCPFC scientific

committee meetings and discussions with the Pacific Community, the Pacific Community are currently considering how sampling undertaken by observers across the WCPFC area might be modified to facilitate progressing the collection of samples that might support such a work program.

Implications

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. The outputs of those discussions will be presented as an information paper to the 2020 meeting of the WCPFC scientific committee and has been identified at the recent SPC Heads of Fisheries meeting held in May 2020 as an area of work that the Pacific Community is committing to. This project and its regular updates to the WCPFC scientific committee meetings have contributed to an overall increasing interest in understanding the population structure of tuna species across the WCPFC region.

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).

This project has contributed to building Australia's leading capability in this field and discussions between CSIRO and the Pacific Community on developing a collaborative programme of work are ongoing. Finally, the project has highlighted that investigations into the population structure of tuna and billfish species utilising the methods detailed here are cost competitive in comparison to current conventional monitoring and assessment methods (e.g. conventional tagging, traditional surveys). They therefore provide financially feasible solutions to resolving a number of problems in fisheries management allowing identification of fish at the level of (i) species, (ii) genetic grouping, and (iii) individual.

Recommendations

The population structure of tuna and billfish species across the western and Central Pacific Ocean is of increasing interest given the potential of genomics using SNPs in resolving questions around structuring and connectivity. Key recommendations from this project are:

1. To work nationally and with regional partners within the WCPFC to collect and analyse samples from a larger set of locations to better understand the population structure and connectivity of target species caught within the western and central Pacific Ocean. Further to work with regional partners more broadly to understand population structure at basin scales and to better understand the connectivity of species between regional fisheries management organisation areas.
2. To continue to work closely with the Tropical Tuna Resource Assessment Group and researchers developing harvest strategies for ETBF species to provide updated information on the connectivity of species caught in the ETBF with those caught in the WCPFC area and using that information, engage where relevant in developing harvest strategies more broadly across the western and central Pacific Ocean.
3. To continue to engage with the Pacific Community and the WCPFC to provide information relevant for further developing sampling programs for the WCPFC Tropical Tuna Tissue Bank that can support population structure studies.

Further development

Issues with accessing samples in adequate numbers from some regions for broadbill swordfish and striped marlin meant that either limited numbers of locations could be included in this study (broadbill swordfish) or that temporal stability in samples was unable to be assessed (broadbill swordfish, striped marlin). Further sampling of both broadbill swordfish from New Zealand and the Cook Islands is planned, with additional internal CSIRO funding committed to analysing these additional samples and establishing the degree of temporal stability of results from these locations.

Development of a broader collaborative body of work on the population structure of tuna species across the western and central Pacific Ocean is a focus of the Pacific Community and CSIRO with an initial design study underway and a funding proposal for facilitating an initial scoping study being developed.

Extension and Adoption

This project would not have been possible without the development of collaborations between operators within the ETBF, researchers across the western and central Pacific Ocean and the WCPFC. At the same time, a number of researchers have contributed to the development of analyses used by this project and have provided essential context in regard to harvest strategies for ETBF species. A full list of those that have assisted with the delivery of this project is provided in Appendix One.

Throughout the lifetime of this project, project staff have regularly engaged with the AFMA ETBF manager and provided regular updates to the TT-RAG, particularly as they developed the program of work for updating the harvest strategies for broadbill swordfish and striped marlin. Copies of all information papers submitted to the WCPFC scientific committee meetings have been provided to the TT-RAG. In association, considerable effort has been placed into ensuring that the TT-RAG understood what questions could be asked of the sampling design included in the project and what conclusions could be made from the outputs of analyses through a series of presentations provided to the TT-RAG in March 2017, March 2019 and July 2019 (see Appendix 3). An update on the project to the TT-RAG was scheduled for late March 2020, however this was cancelled due to the COVID-19 pandemic. Discussions are currently underway with the TT-RAG for presentations to be provided in July 2021 following further analyses of broadbill swordfish samples from the Cook Islands and New Zealand. The TT-RAG was provided with an opportunity to review a draft of this final report and provided useful and constructive feedback that has been incorporated into revisions of the draft.

The additional sampling that was required to be undertaken by this project has also allowed project staff to engage directly with industry and provided direct exchange of information on the project between industry and researchers, particularly in the early stages of the project. This was essential for developing a better understanding of what was possible during the project. The project staff will continue to engage with the TT-RAG as further analyses supported through additional internal funding by CSIRO are conducted.

Engagement with WCPFC and Pacific Community staff in accessing samples from the WCPFC Tropical Tuna Tissue Bank has facilitated ongoing discussions on the development of the WCPFC Tropical Tuna Tissue Bank and greater engagement by project staff in regional planning processes. The guidance provided by this project and provided to the WCPFC has resulted in the Pacific Community developing a draft sampling program for the WCPFC Tropical Tuna Tissue Bank that will be tested over the coming years (COVID-19 pandemic restrictions pending) to support projects focused on population structure studies.

Provision of annual updates on the project to the WCPFC scientific committee have contributed to increasing interest in the development of a program of work investigating the population structure of tuna species in the WCPFC area. They have also assisted in raising the profile of Australia as leaders in this field. An update on the overall results presented in this report was presented to the WCPFC scientific committee in 2020, and project staff will continue to engage with WCPFC where relevant.

An abstract has been submitted to the World Fisheries Congress for a presentation at the conference (now scheduled for 2021 as a result of the COVIC-19 pandemic). At least one peer-review publication is planned on the basis of the results of the project.

Project materials developed

Annual information papers to the WCPFC have been produced throughout the lifetime of the project and a number of presentations provided to the TT-RAG. These have been attached in Appendix Two (WCPFC information papers) and Three (presentations to the TT-RAG)

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Appendices

Appendix One: Contributors to this project

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Appendix Two: WCPFC-SC information papers

Information paper SA-IP-15 presented to the twelfth regular session of the Scientific Committee, 2016.

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

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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 because of the cross-jurisdictional nature of the stocks and governance through Australia's Harvest Strategy Policy and the WCPFC. Current assessments conducted by the WCPFC assume that these species comprise either single discrete stock units throughout the WCPFC area or across the Southern Hemisphere portion of the region. Genetic methods used in the past have been unable to refute such assumptions, however an investigation into the stock structure of yellowfin tuna using next generation sequencing (NGS) methods supports the presence of previously undescribed structure within populations across the Western and Central Pacific Ocean. 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 suggest that populations throughout the WCPFC region may also be structured. Consequently, current assumptions of single spawning populations may not be accurate. 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 NGS technology to improve understanding of the population structure of five species caught in the ETBF (albacore, bigeye and yellowfin tunas, broadbill swordfish and striped marlin) and establish their connectivity with the broader WCPFC region. Results from this project will allow for a re-evaluation of the current paradigm in domestic and regional pelagic fisheries scientific advice and management. The project will provide key information for appropriate governance through the Commonwealth Harvest Strategy Policy domestically and the WCPFC regionally, to ensure any risks to regional stock biomass are minimised and to improve stakeholder concern over stock management.

Introduction

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).

Management of the ETBF is complex because of the cross-jurisdictional nature of the stocks and governance through the Australia's Harvest Strategy Policy and the WCPFC. Current assessments conducted by the WCPFC assume that these species comprise either single discrete stock units throughout the WCPFC area or across the Southern Hemisphere portion of the region and genetic methods used in the past have been unable to refute such assumptions. Biological information on growth rates and reproduction, movement data derived from tagging studies and spatial and temporal variability in catches of these species however, suggest that there is likely to be some structure to stocks throughout the WCPFC region and assumptions of single spawning populations may not be accurate.

More recently, traditional and next generation genomic 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 obvious implications for the management both within national and regional contexts.

Here, we detail a three year project funded through the Fisheries Research Development Corporation on behalf of the Australian Government and the CSIRO that commenced in July 2016. The primary aim of this project is 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. Results from this project should permit a re-evaluation of the current paradigm in domestic and regional pelagic fisheries scientific advice and management. The project seeks to alleviate stakeholder concern over stock management by providing key information for appropriate governance through the Australia's Harvest Strategy Policy domestically and the WCPFC regionally, to ensure any risks to localised stock biomass are minimised.

Methods

Biological sampling

Sampling protocols developed as part of a previous joint Pacific Community (SPC)-CSIRO project have been incorporated into the regional observer program for the WCPFC and a collaborative arrangement between SPC, the Fisheries Forum Agency (FFA), WCPFC and CSIRO and other laboratories across the Western and Central Pacific Ocean is providing for the collection and archiving of biological samples of tropical tuna, billfish and other species across the western Pacific (Nicol et al., 2015).

A spatial assessment of tissue samples for tropical tunas and billfish species held in the WCPFC Tissue Bank and historical samples held by CSIRO has identified key areas where samples are available for stock structure analyses on the five principal species are distributed. Initial discussions with SPC in facilitating access to currently held samples and strategies for collecting samples not currently held in the WCPFC Tissue Bank occurred in early July 2016. Further discussions on the feasibility of extension of current sampling to resolve spatial distribution and species gaps in the Tissue Bank are planned. The CSIRO currently holds an extensive collection of tissue samples from the five species and where there are spatial distribution and species gaps in tissues currently held, we will undertake collection of samples for each of the five species via sampling of fish during

onshore processing. Where relevant, we will also negotiate with recreational fishers for further collection of samples.

Samples derived from three sites will be collated for each of the five species in each of two years (100 per site per year to derive provenance and 50 per site per year to derive mixing). Samples during the first year of sampling will comprise running ripe gonads from spawning fish and will provide for the determination of provenance of spawning. Samples collected in the second year of sampling will comprise a mixture of cohorts and will establish the degree of mixing of fish across the three areas. Specific sample sites for each of the five species will be chosen based on current understanding of spatial structure in populations and availability of samples for analyses. As a result, sites are unlikely to be completely consistent between species.

Genomics and analyses

Variation present at single nucleotide polymorphism (SNP) markers extracted from samples collated from the three sites will be examined using next generation sequencing techniques. This sequencing technology facilitates high throughput/low cost genotyping, allowing firstly verification of species identification, secondly investigation of stock structure/identification of ocean of origin and thirdly, provision of markers useful for close kin approaches to estimating spawning stock biomass (e.g. Bravington et al. In Press). These techniques are currently being used by CSIRO in investigations of the population structure in skipjack, yellowfin, and bigeye tuna throughout the Indonesian archipelago, have provided preliminary evidence of population structure in yellowfin tuna across the Pacific Ocean and form the basis of a broad collaborative project under development for the whole Indian Ocean (see also working paper SA-WP-01). The techniques are proving capable of discriminating population structure where other techniques have failed and are therefore the most suitable technologies for investigating population structure in pelagic species available.

Genomic information from additional outlier sites required for establishing provenance will be derived from other existing genomic projects being conducted by CSIRO across the Indian Ocean. The genomic information derived from the samples analysed under this project and data from the outlier samples derived from other projects will be input into statistical models currently being developed by CSIRO for use in the projects listed above. These models will then be applied to investigate the presence and level of stock structure across the five species.

Outputs and outcomes

The project will provide a comprehensive evaluation of the population structure and connectivity of the five principle species targeted in the ETBF. Working papers and presentations will be provided to national and regional fisheries management agencies and papers for publication in peer-review journals will be developed.

The improved understanding of stock structure will enable a re-evaluation of the current paradigm 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. Furthermore, understanding of the spatial dynamics of each species will allow Australia (and neighbouring coastal states) to understand and assert their property rights within the WCPFC management framework. In the Australian domestic context, this will allow for the updating of the harvest strategy current used in the management of the ETBF with operating models that have increased accuracy and precision. If a revised and more detailed population structure emerges from the genomic work conducted under this project, close-kin mark-recapture estimators and feasible sampling regimes can be developed.

Once implemented, these estimators should reduce uncertainty in spawning stock size, and could potentially provide an abundance index for use in harvest strategies.

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

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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 NGS 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 progress on the first year of the project, including preliminary testing of historical samples held by CSIRO and the WCPFC Tuna Tissue Bank and collection of contemporary samples throughout the ETBF and western Pacific Ocean.

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, traditional and next generation genomic 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 obvious implications for the management 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 these techniques and associated protocols for sample handling, quality control and processing in conjunction with a specialised processing laboratory based in Australia.

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) 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 and central 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.

Progress to date

Muscle samples analysed by the project will comprise historical collections held by the CSIRO and the WCPFC Tuna Tissue Bank, and where samples from historical collections are inadequate, contemporary collection of muscle tissue will occur where feasible. The aim of the project is to be able to compare variability present at single nucleotide polymorphism (SNP) markers in the five species from three locations: the ETBF and two across the western and central Pacific Ocean (see Evans et al. 2016).

In order to determine the locations from which samples were to be compared 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. This survey has identified availability of tissues and their partitioning among key sampling sites as well as potential areas that sampling might be focused on for use in examining stock structure of each of the species. Initial planned spatial sampling structure for the project is provided in Table 1.

Discussions with staff from the Oceanic Fisheries Program (OFP) of the Pacific Community have focussed on facilitating access to samples currently held in the WCPFC Tuna Tissue Bank, with an application submitted to the WCPFC Secretariat (currently under consideration) to access samples from albacore, bigeye and yellowfin tunas .

Table 1. Proposed sampling structure for investigating the population structure of five tuna and billfish species.

Albacore tuna	Bigeye tuna	Striped marlin	Swordfish	Yellowfin tuna
ETBF	ETBF	ETBF	ETBF	ETBF
Fiji	Fiji	New Zealand	Fiji	Fiji
Cook Islands/French Polynesia	Solomon Islands	Cook Islands/French Polynesia	Cook Islands/French Polynesia	Solomon Islands

Initial testing of historical samples

As a first step in investigating the viability of those historical samples identified and held by CSIRO and in the WCPFC Tuna Tissue Bank (provided by OFP staff), a quality control sequencing trial of samples was run in late 2016/early 2017. All samples from the WCPFC Tuna Tissue Bank passed quality control checks with both target quality control and single nucleotide polymorphism distance matrix results provided to OFP staff. Overall results from CSIRO collections were also positive, with the majority passing quality control checks.

Collection of contemporary tissue samples

Collection of contemporary samples from the ETBF was initiated in late 2016 with a first year of swordfish and bigeye tuna samples collected. Sampling of striped marlin within the ETBF via both commercial and recreational fishers has also commenced. Access to striped marlin samples from the New Zealand region is being facilitated through Blue Water Marine Research, with sampling planned to begin in September 2017.

A table of all samples currently held, those in the WCPFC Tuna Tissue Bank access has been applied for and those planned to be collected are provided in Table 2.

Next steps

Samples from albacore tuna, striped marlin and swordfish from the ETBF are currently being sequenced. Further collection of samples from bigeye tuna, striped marlin and swordfish are ongoing both in the ETBF and in New Zealand for striped marlin. As samples are collated, they will also be submitted for DNA sequencing. Initial runs of specialised models developed by CSIRO for discriminating stock structure will commence in the first half of 2018.

Discussions examining strategies for collecting samples from target areas for the project where there are currently not suitable numbers of samples in the WCPFC Tuna Tissue Bank (Solomon Islands, Fiji, Cook Island/French Polynesia) have been initiated with staff from the OFP. The authors would also like to use this meeting to extend these discussions to other relevant agencies across the WCPFC area on the feasibility of collecting samples from:

(i) bigeye tuna from the Solomon Islands region;
(ii) albacore and swordfish from the Fiji region;
(iii) albacore tuna, striped marlin and swordfish in the Cook Islands/French Polynesian region

Intended outcomes

The improved understanding of stock structure 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.

Table 2. Status of sampling from the five tuna and billfish species

Species	Location	Years	Status
Albacore tuna	ETBF	2	collection completed
	Fiji	1 (1)	WCPFC application submitted (planning underway)
	Cook Islands/French Polynesia		planning underway
Bigeye tuna	ETBF	2	collection underway
	Fiji	2	WCPFC application submitted
	Solomon Islands		planning underway
Striped marlin	ETBF	2	collection underway
	New Zealand	2	collection underway
	Cook Islands/French Polynesia		planning underway
Swordfish	ETBF	2	collection underway
	Fiji		planning underway
	Cook Islands/French Polynesia		planning underway
Yellowfin tuna	ETBF	2	collection complete
	Fiji	2	WCPFC application submitted
	Solomon Islands	2	WCPFC application submitted

Reporting

We anticipate providing updates on the project in the form of information papers to the WCPFC scientific committee at their meetings in 2017, 2018 and 2019, with further information on project progress provided to WCPFC if and when needed. 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.

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

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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

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

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Abstract

This paper provides an update on a three-year project investigating the connectivity of tuna and billfish species caught in the fishery that operates along the east coast of Australia with those in adjacent waters and those further east in the Pacific Ocean. The project is funded through the Fisheries Research Development Corporation on behalf of the Australian Government and the CSIRO. Samples examined for this study came from three sources: 1. the Western and Central Pacific Commission's Tropical Tuna Tissue Bank (from New Caledonia, Fiji and the Marshall Islands); 2. historical samples collected by CSIRO and Indonesia (from Australia, New Zealand and the Solomon Islands); and 3. contemporary samples collected by CSIRO, independent researchers and industry from Australia and New Zealand. DNA extractions were assayed using double digest RAD sequencing techniques followed by quality assessment incorporating a multi-stage Quality Control (QC) analysis approach. A step-wise quality control analysis of albacore and yellowfin tuna sequencing data revealed varying levels of DNA cross contamination across all sampled locations. Following quality control analysis data from samples of sufficient quality (at numbers allowing for rigorous population analysis) were input into mixture models. Model results failed to provide evidence for multiple populations across the sampled regions for both species. Based on our results, we discuss potential for future analyses and suggestions for utilising tissues sampled from the WCPFC Tropical Tuna Tissue Bank in the context of genetic studies.

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; 2018) 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). 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 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 (see Table 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 Takayama 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 (as opposed to an admixture model) was based on the method outlined in Foster et al. (2018) and implemented in the package stockR (Foster 2018). The model assumes that each sample belongs to one of K populations ($K \geq 1$ and is an integer), and the purpose of the analysis is twofold: 1. to choose K and, 2. to assign each sample to one of the populations.

Three approaches are utilised in order to determine the best fit to the model given varying values of K:

1. Information Criteria: Two information criteria (AIC and BIC) are calculated from the fitted model with the number of stocks (K) that minimised the information criterion identified as providing the best fit.
2. Cross Validation: 5-fold cross validation was used to evaluate how quickly the predictive performance of the model diminished as more stocks were added. To obtain the cross-validation statistics B = 1000 holdout samples were used.
3. Bootstrapped Confidence Intervals: confidence intervals associated with group membership increase as the model become over-fitted and the certainty to which population a sample is assigned decreases. To obtain confidence intervals B = 1000 bootstrap samples were used.

Progress to date

The spatial sampling structure for the project and samples included in the project based on historical and contemporary collection of samples and the current state of collection and analysis is provided in Table 1. Given the nature of the collections from which samples were derived, samples comprised a mix of sexes, lengths (albacore: 48 – 100cm, bigeye: 31 - ~150 cm, yellowfin: 88 - ~150 cm; striped marlin and swordfish yet to be completed) and therefore age classes/cohorts and potentially reproductive state. Full analysis of albacore and yellowfin tunas has been completed and will be presented here. Remaining species analysis is yet to be completed.

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

Examination of gel runs on DNA extracted from yellowfin tuna samples initially received from the WCPFC Tuna Tissue Bank identified 49 samples that had degraded to the point that very little high molecular weight DNA could be extracted, which is necessary for the DArTseq™ technique. As a result, replacement of poor quality individuals by others in the WCPFC Tuna Tissue Bank was required. 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. The catch location of a further one sample was unable to be confirmed by Pacific Community staff. As a result, 50 samples did not progress to genetic sequencing (see Table 1).

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 and sequenced
Bigeye tuna	ETBF	2	collected and sequenced y
	Marshall Islands	2	collected and sequenced
	Solomon Islands	2	collected and sequenced
Striped marlin	ETBF	2	collected, sequencing underway
	New Zealand	2	collected and sequenced
	Hawaii	2	collected, sequencing underway
Swordfish	ETBF	2	collected and sequenced
	NZ	1	collection underway
Yellowfin tuna	ETBF	2	collected and sequenced
	Fiji	2	collected and sequenced
	Marshall Islands	2	collected and sequenced

Species identification

Species identification identified six samples where species had been misidentified or mislabelled. Four samples of yellowfin tuna from the Marshall Islands were misidentified/mislabelled as bigeye tuna and two samples of bigeye tuna from the Marshall Islands were misidentified/mislabelled as yellowfin tuna. All misidentified/mislabelled samples were derived from different sampling events.

Quality control of sequencing data

The quality control steps at which samples were removed and the numbers removed at each step are detailed in Table 2.

Missing data

If an individual is missing data above a threshold as a result of poor-quality DNA, they are removed. A total of 16 albacore tuna samples from the ETBF, six albacore tuna samples from New Zealand, eight yellowfin tuna samples from the ETBF and one yellowfin tuna sample from Fiji were removed at this step.

Genome wide average heterozygosity

The position at which a SNP occurs on a chromosome is called the locus (plural loci). Because SNPs are bi-allelic, they contain two alleles at each locus. Heterozygosity is a measure of how many loci contain two different alleles (heterozygous genotype) versus how many loci have two identical alleles (homozygous genotype). On average individuals within a population will have the same level of heterozygosity as each other. However, if the heterozygosity observed for the DNA profile of an individual deviates from this average then this likely reflects sample cross contamination – introduced 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). Conversely, samples with lower than average heterozygosity are likely an indication of poor DNA quality that results in a homozygous excess as a result of introduced artefactual sequencing bias. An important step in assessing the quality of samples is therefore to identify samples that are either too homozygous or too heterozygous compared to the average observed level of heterozygosity. To do this, the level of genome-wide mean heterozygosity is calculated. For the current study, individual samples with a mean heterozygosity above and below statistical threshold values of higher and lower confidence limits and are filtered out of datasets for further quality control.

Of albacore tuna samples, a total of 22 samples from the ETBF, 30 samples from New Caledonia and ten samples from New Zealand were removed due to above average heterozygosity. Of yellowfin tuna samples, a total of 15 samples from the ETBF, 45 samples from Fiji and five samples from the Marshall Islands were removed at this step (Table 2).

Similar genotypes

Genetic similarity is used to identify individuals that are closely related where more closely related individuals show higher levels of genetic similarity and by extension show lower levels of genetic distance between them relative to average genetic distance between unrelated pairs. In essence, non-related individuals should have genotypes that are dissimilar (because they have no common relatives to derive their genes from). However, when cross-contamination or technical mishaps occur (e.g. labelling two samples collected from the same individual as different animals), samples with similar or almost identical genotypes can occur among individuals sampled from a population. Care needs to be taken in examining individuals with similar genotypes to determine if values of genetic distance are reflective of relatedness or the result of human error.

In addition, the sequencing process includes a number of technical replicates. These are included to examine the repeatability of sequencing results and so therefore need to be removed prior to any further analysis of sequencing results.

Of samples sequenced, the majority of individuals identified as having similar genotypes were either technical replicates or replicate samples (included to examine potential differences caused by the two different extraction methods). Of those remaining, four albacore tuna samples from the ETBF, and four yellowfin tuna samples, two from Fiji and two from the Marshall Islands were removed. Examination of the genetic distance between pairs and the metadata associated with each identified that these were unlikely related individuals, but rather similarities were caused by either cross contamination or human error (e.g. spreadsheet/database errors or sample mislabelling).

Population modelling

For the two species that analysis has been finalised (albacore and yellowfin tuna) none of the three methods utilised could confirm the presence of more than one genetic population present in samples from ETBF and the two other sites investigated for each species (Figures 1 and 2). The two information criteria examined (AIC and BIC) were minimised at a value of K = 1, cross validation was capable of assigning all samples at K = 1 and at higher values of K rapidly declined and confidence intervals associated with group membership increased substantially at values of K > 1. This suggests a level of connectivity between and mixing of fish that are caught in the three areas that results in little discernible genetic differentiation by the approach utilised here. It must be noted that these results *only apply to the three sites* included in this study and therefore *cannot* be extrapolated across the wider western Pacific Ocean region.

Samples from bigeye and yellowfin tunas from a larger number of sites are currently the subject of ongoing investigations under a number of projects across the Indian and Pacific Oceans by the CSIRO and collaborating institutions and agencies (see Grewe et al. 2019). Results to date suggest that both bigeye and yellowfin data demonstrate genetic differentiation across an area extending from the central Indian to Eastern Pacific Ocean region and indicate that at these larger scales (larger than those examined in this study), some degree of restriction to genetic connectivity exists.

The mixed nature of the samples defined 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 determine if multiple spawning populations for 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 a period from which temporal stability in results could be confirmed.

Next steps

Full analysis of sequencing data from bigeye tuna samples is underway. The final samples of striped marlin and swordfish are currently being collected and prepared for sequencing. Once sequenced, data will be analysed similarly to those samples presented here.

Table 2. Samples genotyped and removed through the species identification and quality control processes.

Species	Region/EEZ	Year	Number samples received	Number genotyped (incl. replicates)	DNA quality (qualitative)	Species ID	Missing data	Heterozygosity	Similar genotypes
Albacore	Australia	2009	50	73	—	—	16	1	15 (all technical replicates)
		2010	50	50	—	—	6	—	—
Albacore	New Caledonia	2013	25	36	—	—	—	1	8 (all technical replicates)
		2014	37	44	—	—	—	—	7 (all technical replicates)
		2016	45	64	—	—	—	30	10 (all technical replicates)
Albacore	New Zealand	2008	47	63	—	—	6	5	14 (12 technical replicates)
		2010	47	47	—	—	—	5	2
Yellowfin	Australia	2006	52	65	—	—	8	15	9 (all technical replicates)
		2013	50	77	—	—	—	—	33 (all replicate samples or technical replicates)
Yellowfin	Fiji	2014	62	77	9	—	—	30	14 (13 technical replicates)
		2015	60	39	25		1	15	3 (two technical replicates).

Species	Region/EEZ	Year	Number samples received	Number genotyped (incl. replicates)	DNA quality (qualitative)	Species ID	Missing data	Heterozygosity	Similar genotypes
Yellowfin	Marshall Islands	2014	63	51	13	3	—	3	2 (one technical replicates)
		2015	52	60	2	1	—	2	10 (nine technical replicates)

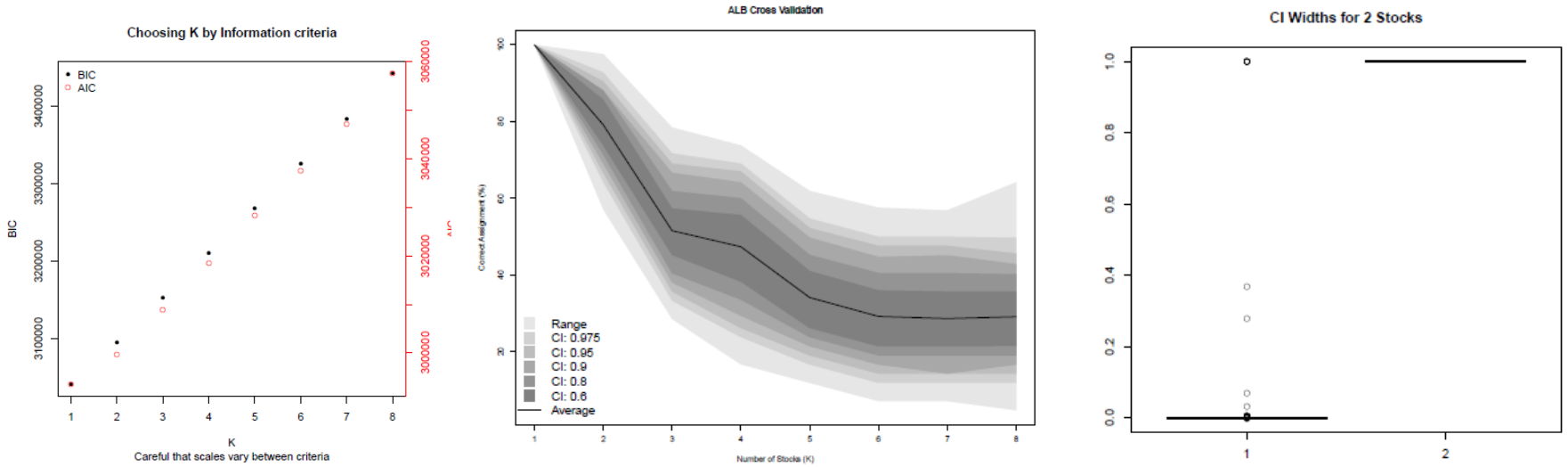

Figure 1. Model results for albacore tuna across the three approaches utilised. L-R: information criteria, cross validation, confidence intervals.

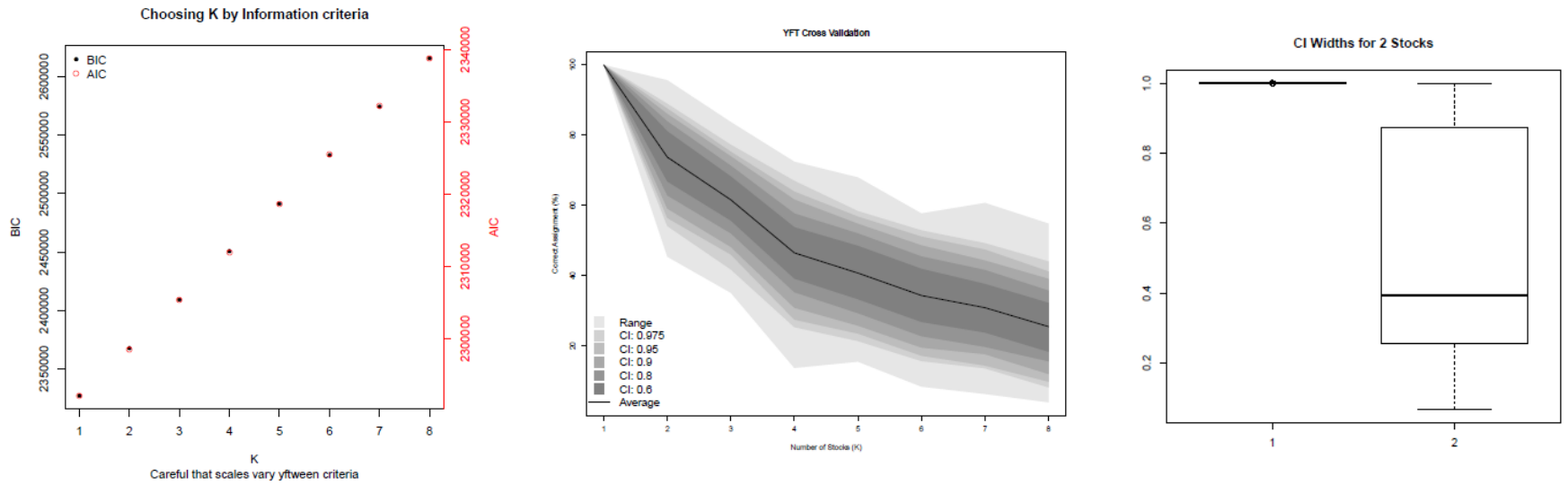

Figure 2. Model results for yellowfin tuna across the three approaches utilised. L-R: information criteria, cross validation, confidence intervals.

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 and 2019. We anticipate providing the WCPFC scientific committee with results from the project in the second half of 2019. A final report will be produced for submission to the Australian Government Fisheries Research Corporation in the second half of 2019 and an associated peer review publication produced.

Intended outcomes

The information provided by this project will enable improved population structure and mixing 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.

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

Large scale investigations focused on establishing the presence of stock structure across fisheries 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) Identification 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 Tropical 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 Tropical 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 Tropical 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 investigations of species stock structure and population connectivity across the WCPFC:

V. Species coverage

Tissues currently contained in the WCPFC Tropical 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. shark species). 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 and population connectivity.

VI. Spatial coverage

Tissues currently contained in the WCPFC Tropical Tuna Tissue Bank to some extent reflect the distribution of the highest catches across the WCPFC Area. As a result, there are particular regions where samples are almost 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 and population connectivity.

VII. Sample sizes

Tissues currently contained in the WCPFC Tropical Tuna Tissue Bank, whilst impressive overall, rapidly decline in numbers once distributed on the basis of species, sample type and spatial and temporal qualifiers. In particular, the utility of the Tissue Bank declines for stock structure and population connectivity 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 and then across a number of years. This declines further when attempting to target samples from particular year classes or cohorts and align samples with gonad samples for the identification of spawning individuals. Greater focus on building tissue samples from a small number of regions across multiple years (these regions could vary through time) and from particular age classes and/or reproductive state would substantially increase the utility of the Tissue Bank for stock structure and population connectivity studies.

VIII. Quality of samples

The quality of samples included in this study varied considerably with misidentification/mislabelling of species, samples of low DNA quality and cross-contamination identified across datasets. The incidence of these factors has flow on effects on overall sample numbers for analysis and highlights the need for first, strict sample collection and handling protocols and second, appropriate data quality control processes to be factored into studies. Without strict quality control measures, particularly those that allow for the identification of cross contamination, the potential for misinterpretation of data is increased. In this study sample sizes were reduced by as much as 53 individuals in any one year (yellowfin tuna from Fiji). Such large reductions in sample sizes reduce the robustness of any analyses, reducing confidence in results and have flow on impacts on the applicability of sample archives such as the WCPFC Tropical Tuna Tissue Bank for stock structure and population connectivity studies.

Acknowledgements

The authors would like to thank the members of WCFPC 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 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 and Heidi 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.

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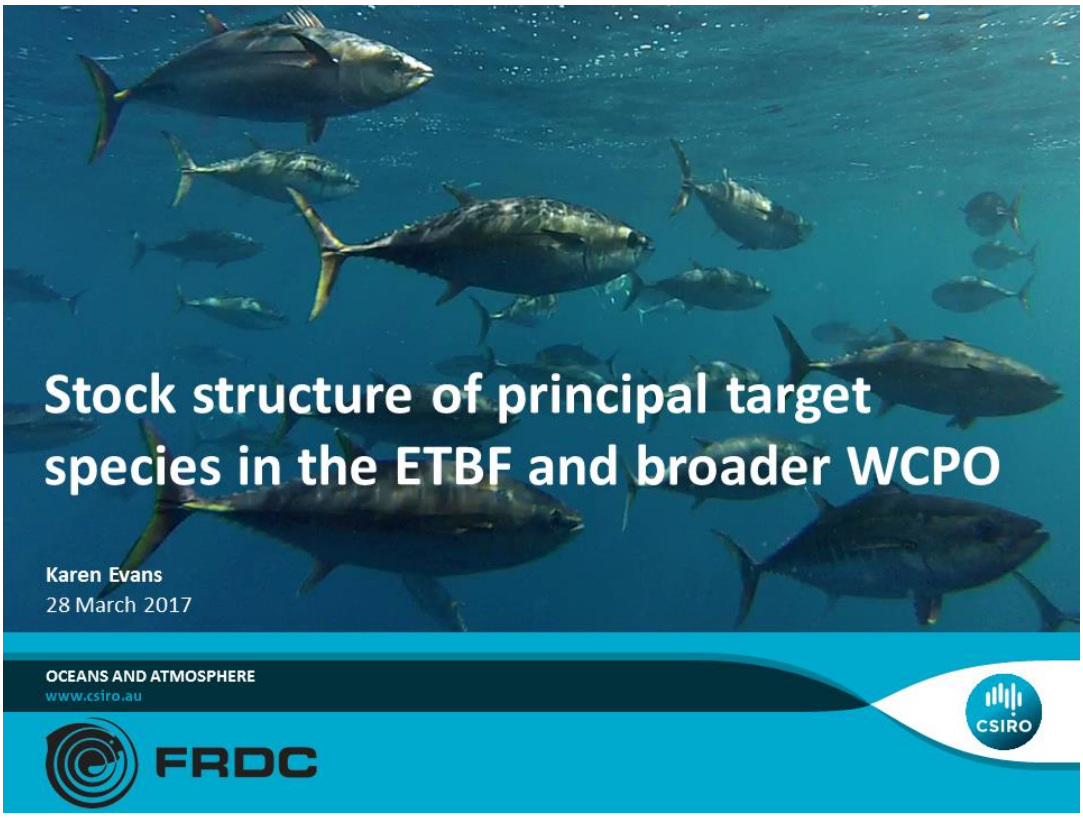
Appendix Three: Presentations to the TT-RAG

March 2017



Addressing uncertainties

Reviews of data to date suggest there is some degree of residency of the five main species captured in the ETBF

Population structure across all five species probably higher than previously thought

Previous FRDC-CSIRO and AFMA-CSIRO projects have identified that:

- uncertainty in spatial connectivity would impact the future performance of the current management approach
- reducing uncertainty in stock structure was of particular priority
- the most cost effective and appropriate methods for reducing uncertainty were next generation genomic methods

Investigating stock structure and mixing

FRDC-CSIRO project: "Determination of the spatial dynamics and movement rates of the principal target species within the ETBF and connectivity with the broader WCPO – beyond tagging" 2016-2019

Objectives:

1. Investigate the presence of stock structure in the five principal species caught in Australia's Eastern Tuna and Billfish Fishery and the western Pacific Ocean across spatial scales of relevance using new generation genomic methods
2. Assess the need and associated costs for research required to further reduce uncertainties in stock structure relevant harvest strategies and management frameworks
3. Inform the relevant parties in the Western and Central Pacific Fisheries Commission of the key results and, if appropriate, the need and value of extending the project throughout the western and central Pacific Ocean.

Investigating stock structure and mixing

Methods

Variation present at single nucleotide polymorphism (SNP) markers from tissue samples from three sites (one ETBF, two external) for each species will be examined using NGS techniques

Sites informed by current knowledge, vary between species

Samples a mixture of locally sourced (ETBF), WCPFC Tissue Bank and directly sourced (ETBF and WCPO)

50 samples from each of the three sites in each of 2 years (100 samples total)

Progress: samples

First step: establish the distribution of samples for each species across historical datasets

- enabled identification of potential sites
- identification of species and areas requiring targeted sampling
- some initial negotiation with University of the South Pacific to minimise overlap between the two projects

Species	Site 1	Site 2	Site 3
Albacore	ETBF	Fiji	Cook Is/French Polynesia
Bigeye	ETBF	PNG/Solomon Is	Fiji
Striped marlin	ETBF	Fiji	Cook Is/French Polynesia
Swordfish	ETBF	Fiji	Cook Is/French Polynesia
Yellowfin	ETBF	PNG/Solomon Is	Fiji

ETBF samples (CSIRO held)

Species	Year: sample number
Albacore	2007: 39 2009: 254 2010: 4
Bigeye	2006: 4 2009: 3
Striped marlin	1996: 34 2006: 6 2016: 13*
Swordfish	2006: 50 2016: 101*
Yellowfin	2006: 82 2009: 11 2008: 79 2013: 94

*Far eastern ETBF

WCPO samples (CSIRO held)

Species	Site, Year: sample number
Albacore	New Zealand 2010: 62
Bigeye	Solomon Islands 2013: 75 2014: 52
Striped marlin	
Swordfish	
Yellowfin	Solomon Islands 2013: 99 2014: 122

WCPO samples (WCPFC Tissue Bank)

Species	Site 2 Year: sample number	Site 3 Year: sample number
Albacore*	Fiji 2008: 1; 2013: 56; 2014: 28; 2015: 30; 2016: 9	Cook Is/French Polynesia 2002: 6; 2003: 8; 2004: 26; 2005: 2; 2009: 5; 2012: 4; 2013: 13; 2014: 4; 2015: 14; 2016: 17
Bigeye*	PNG/Solomon Is 2004: 3; 2005: 13; 2006: 1; 2007: 5; 2008: 3; 2009: 5; 2011: 3; 2012: 22; 2013: 37; 2014: 10; 2015: 8; 2016: 14	Fiji 2003: 2; 2007: 26; 2008: 16; 2009: 8; 2010: 20; 2013: 42; 2014: 21; 2015: 40; 2016: 23
Striped marlin	Fiji 2007: 4; 2009: 1; 2010: 1; 2014: 2	Cook Is/French Polynesia 2002: 4; 2003: 4; 2004: 5; 2009: 3; 2010: 2; 2013: 2; 2015: 2; 2016: 3
Swordfish	Fiji 2008: 1; 2010: 3; 2015: 3	Cook Is/French Polynesia 2002: 4; 2003: 4; 2004: 5; 2009: 3; 2010: 2; 2013: 2; 2015: 2; 2016: 3
Yellowfin*	PNG/Solomon Is 2004: 25; 2005: 25; 2006: 186; 2007: 658; 2008: 93; 2009: 150; 2010: 25; 2011: 113; 2012: 143; 2013: 218; 2014: 125; 2015: 184; 2016: 197	Fiji 2007: 15; 2008: 1; 2010: 31; 2013: 71; 2014: 62; 2015: 100, 2016: 44

Progress: samples

Next step was to establish the viability of historically held samples (aged > 5 years and up to 20 years)

Mixture of samples from CSIRO & WCPFC Tissue Bank samples across all five species

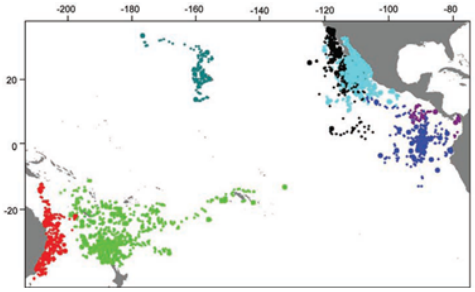
Majority of samples passed QC – only samples to fail were ones identified as potentially unviable

Samples sequenced:

Yellowfin: ETBF (1 year)

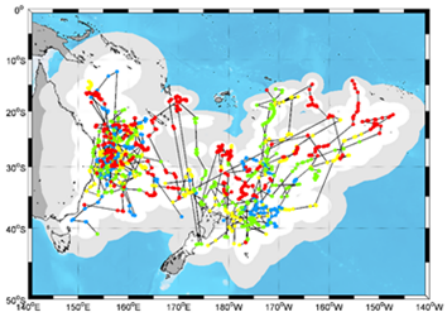
Next steps: samples and sequencing

Currently discussing targeted sampling of albacore, bigeye, striped marlin and swordfish in Fiji and Cook Islands/French Polynesia with SPC



Investigating potential access to striped marlin via recreational fishers in NZ – looking to TTRAG for best contacts for Australia

May need to do the same for swordfish



Next steps: samples and sequencing

Submitting application to WCPFC for access to Tissue Bank samples

Sequencing albacore, swordfish and yellowfin samples on hand

Risks identified at this point:

- accessing striped marlin samples from WCPO (low and dispersed catches); may only have two sample sites (ETBF/NZ)
- accessing albacore samples in adequate numbers from Cook Is/FP; may consider using NZ/eastern New Caledonia samples

Outputs

Working papers and presentations to the TTRAG, TTMAC, DAWR, AFMA and other relevant agencies

Working papers to the WCPFC-SC and for wider distribution to the FFA, PNA, WPRMC

Peer-review publications

Final report to Fisheries Research Development Corporation

Project team

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Rich Hillary
Dale Kolody
Matt Lansdell

Other key collaborators:
Neville Smith, Francois Roupsard, Caroline Sanchez (SPC)
Gary Heilmann, Pavo Walker (ETBF fishery)

Thank you

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March 2019

Connectivity of the principal target species within the Eastern Tuna and Billfish Fishery with the broader western and central Pacific Ocean: project update

Karen Evans, Scott Foster, Pete Grewe | TT-RAG meeting
27 March 2019

OCEANS AND ATMOSPHERE
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Objectives

- Investigate the presence of stock structure in the five principal species caught in Australia's Eastern Tuna and Billfish Fishery and the western Pacific Ocean across spatial scales of relevance using new generation genomic methods
- Assess the need and associated costs for research required to further reduce uncertainties in relevant harvest strategies and management frameworks
- Inform the relevant parties in the Western and Central Pacific Fisheries Commission of the key results and, if appropriate, the need and value of extending the project throughout the western and central Pacific Ocean.

Samples

Historical samples held by CSIRO – ETBF, NZ, Solomon Islands

Samples contained in the Western and Central Pacific Fisheries Commission Tissue Bank

Samples collected specifically for the project - ETBF

Species	Location	Year	Number	Comments
Albacore tuna	ETBF	2009	50	
	ETBF	2010	50	
	New Zealand	2008	50	
	New Zealand	2010	50	
	New Caledonia	2013	27	
	New Caledonia	2014	37	
	New Caledonia	2016	40	
Broadbill swordfish	ETBF	2016	45	
	ETBF	2017	48	
	New Zealand	2019		Collection underway
Bigeye tuna	ETBF	2017	50	
	ETBF	2018	50	
	Marshall Islands	2014	50	
	Marshall Islands	2015	50	
	Solomon Islands	2013	50	
	Solomon Islands	2014	50	
Striped marlin	ETBF	1996	36	
	ETBF	2018	50	
	New Zealand	2018	50	
	New Zealand	2019		Collection underway
Yellowfin tuna	ETBF	2006	50	
	ETBF	2013	50	
	Fiji	2014	51	
	Fiji	2015	36	
	Marshall Islands	2014	50*	
	Marshall Islands	2015	49*	

Methods

Samples sub-sampled – exterior removed

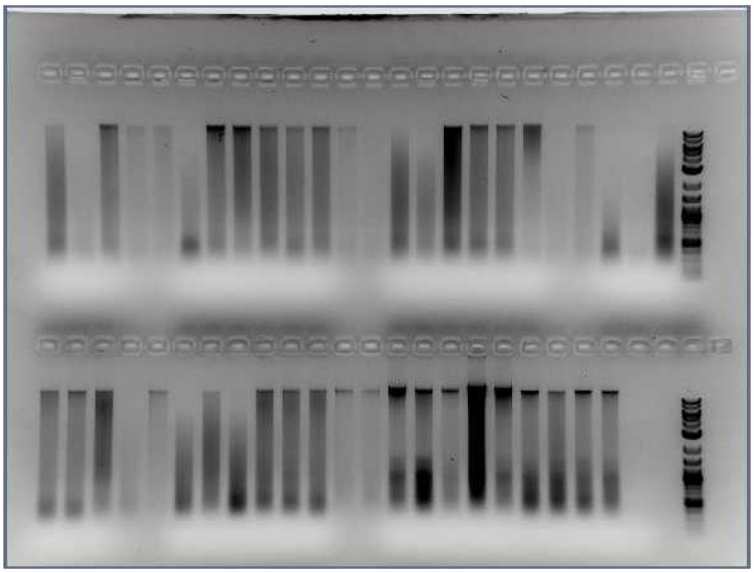
DNA extracted and quality examined (gel and nanodrop)

Samples genotyped, sequenced and SNP markers called at Diversity Arrays Technology (Canberra)

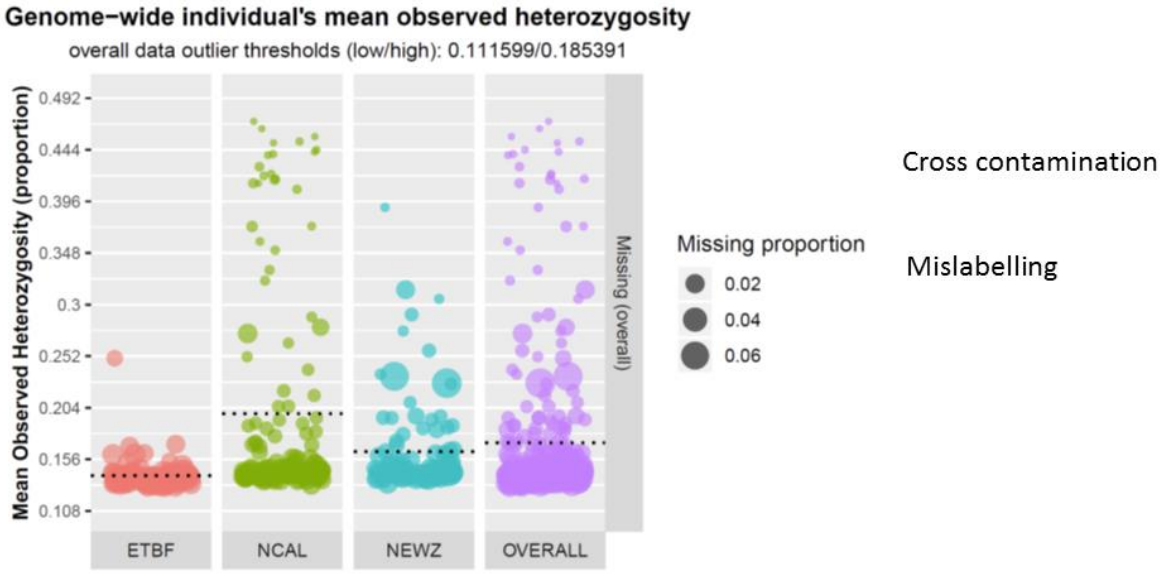
SNP markers run through a series of data QC processes (Radiator)
- filters out samples with low allele frequency and high heterozygocity

Filtered ("clean") SNP markers run through mixture model (StockR)
- identifies number of stocks based on a maximum likelihood solution
- assigns each fish to the number of stocks through a cross validation process

Samples: QC



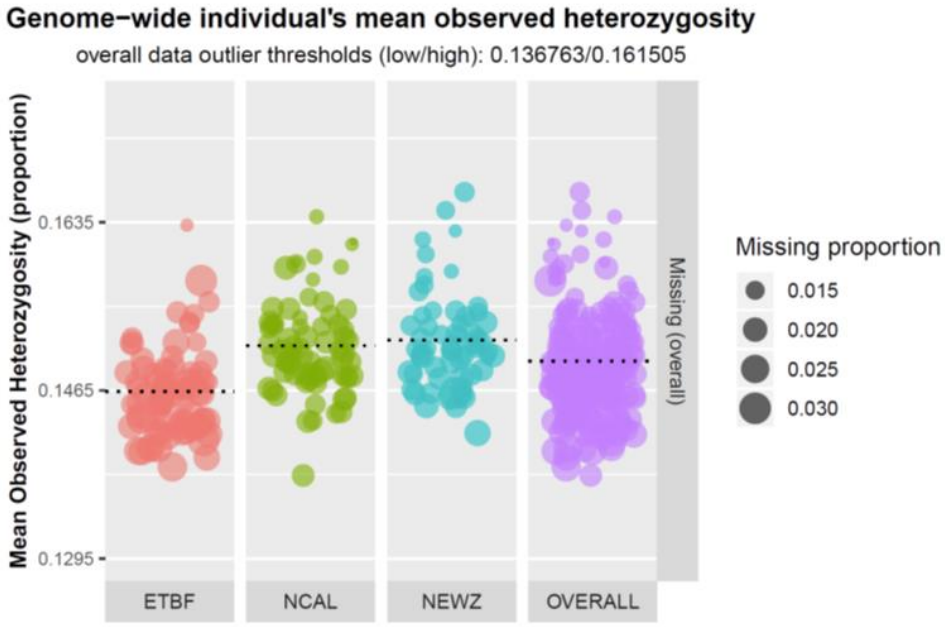
Albacore: QC



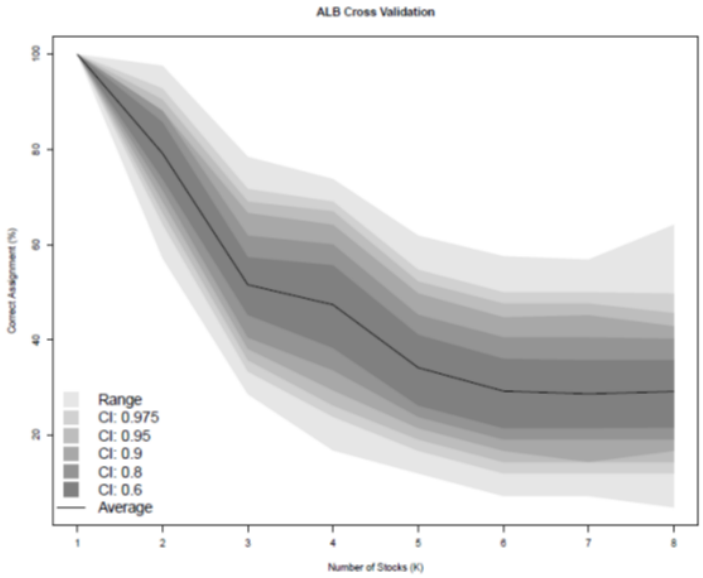
Cross contamination

Mislabelling

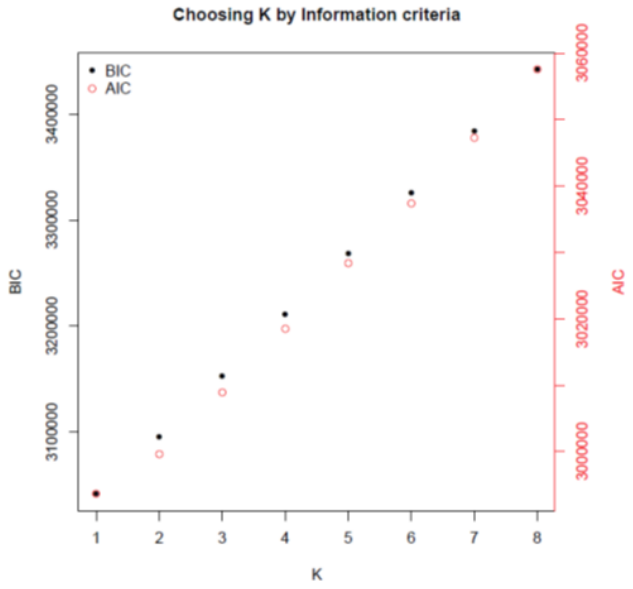
Albacore: QC



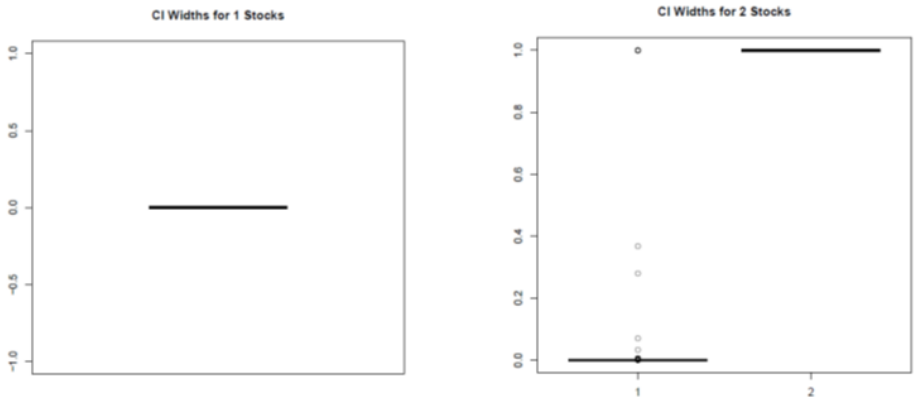
Albacore: model assignment



Albacore: model assignment



Albacore: model assignment



Preliminary interpretation of results

Data do not support the hypothesis that there are multiple stocks present in samples from ETBF, NZ and NC

Things to note:
- samples consist of fish of multiple ages
- samples consist of multiple years
- samples collected across multiple months within a year

In an ideal world* you would sample fish from multiple locations at the same time and sample fish that are spawning

Without the WCPFC Tissue Bank and CSIRO historical samples this project would not exist

* an ideal world rarely exists and is usually super expensive ($$, time, resources) to create

Next steps

Currently running yellowfin and bigeye samples through Radiator
- anticipate having preliminary results in April

NZ swordfish sampling underway

Second year of striped marlin sampling almost complete

Full preliminary results for presentation to WCPFC-SC (August)
- run through with TT-RAG at July meeting

Draft final report submitted end of August

Final report submitted December

Thank you

Project team:
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Matthew Lansdell

With assistance from:
Don Bromhead (AFMA)
Thierry Gosselin (IML/ISMER)
Gary Heilmann and the team at De Brett Seafoods
John Holdsworth (Bluewater Marine Research, NZ)
Francois Roupsard (SPC)
Andy Smith (Talley's Group Limited, NZ)
Neville Smith (SPC)
Heidi and Pavo Walker and the team from Walker Seafoods

July 2019

Connectivity of the principal target species within the Eastern Tuna and Billfish Fishery with the broader western and central Pacific Ocean: project update

Karen Evans, Pete Grewe | TT-RAG meeting
18 July 2019

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Scott Foster, Rasanthi Gunasekera, Matt Lansdell, Walker Seafoods, John Holdsworth, WCPFC, SPC

Objectives

- Investigate the presence of stock structure in the five principal species caught in Australia's Eastern Tuna and Billfish Fishery and the western Pacific Ocean across spatial scales of relevance using new generation genomic methods
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Samples

Historical samples held by CSIRO – ETBF, NZ, Solomon Islands

Samples contained in the Western and Central Pacific Fisheries Commission Tissue Bank

Samples collected specifically for the project – ETBF, NZ

Samples

Albacore

Region/EEZ	Year	Number samples received
Australia	2009	50
	2010	50
New Caledonia	2013	25
	2014	37
	2016	45
New Zealand	2008	47
	2010	47

Yellowfin

Region/EEZ	Year	Number samples received
Australia	2006	52
	2013	50
Fiji	2014	62
	2015	60
Marshall Islands	2014	63
	2015	52

Bigeye

Region/EEZ	Year	Number samples received
Australia	2017	50
	2018	50
Marshall Islands	2014	50
	2015	50
Solomon Islands	2013	72
	2014	49

Samples

Samples are a mix of:

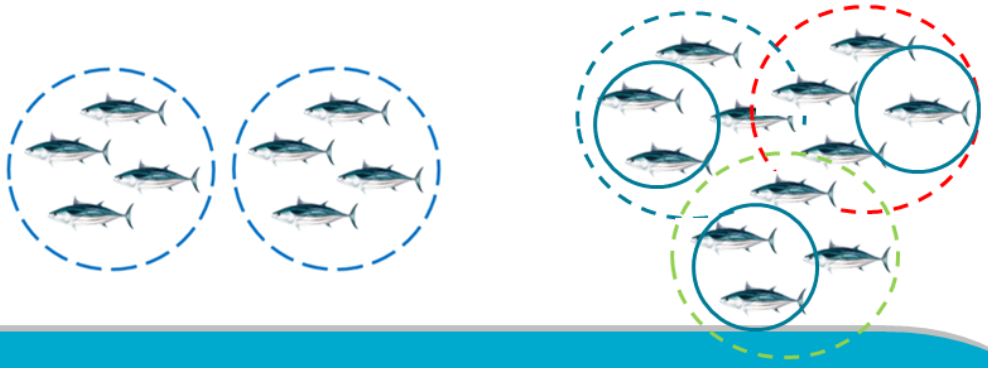
Sexes

Lengths

- age groups/cohorts/year classes

Reproductive state

Composition of samples determine the questions that can be asked

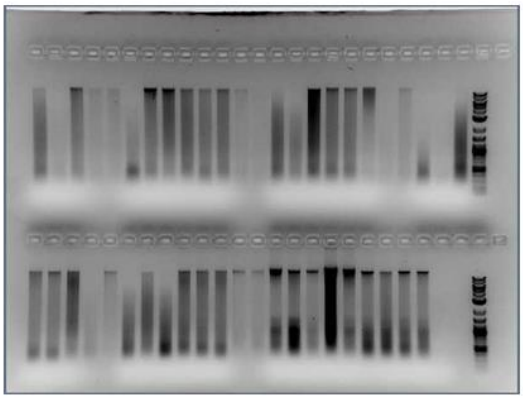


Methods – subsampling and sequencing

Samples sub-sampled – exterior removed

DNA extracted and quality examined (gel and nanodrop)

Samples sequenced and SNP markers called at Diversity Arrays Technology (Canberra)



Quality control processes

Check of species ID

DArT data run through a 15-step quality control process (Radiator)
- filters out data at both the marker and sample levels

Sample filtering occurs at three steps
1. missing data
   - poor quality samples (statistical check on top of visual check of gels)

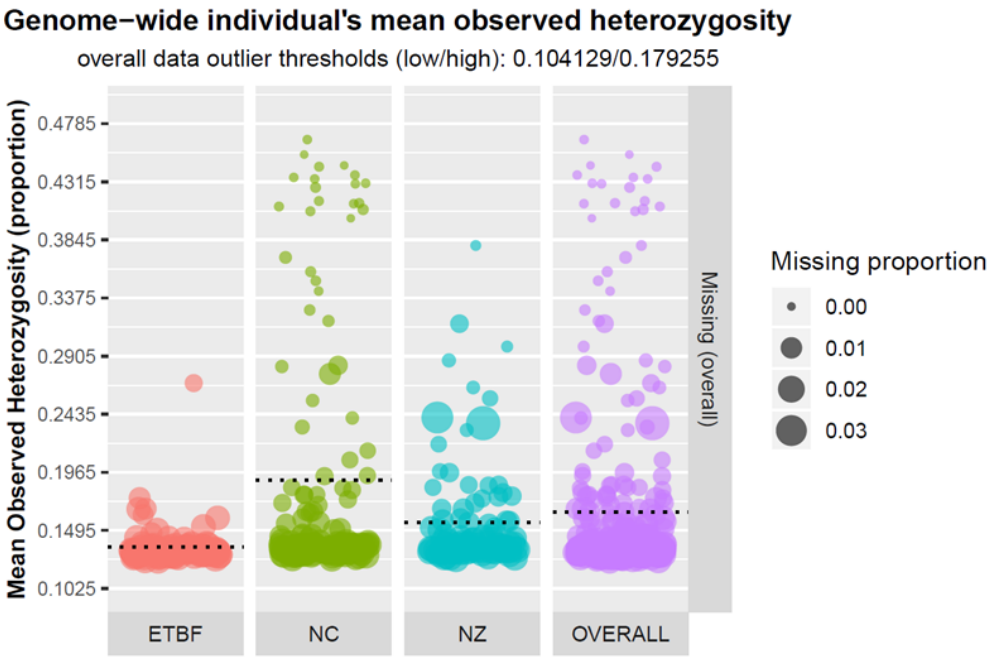
2. degree of heterozygosity
   - cross contamination

3. degree of similar genotypes
   - technical replicates
   - mislabelling of samples
   - cross contamination ("weird Johnnies")

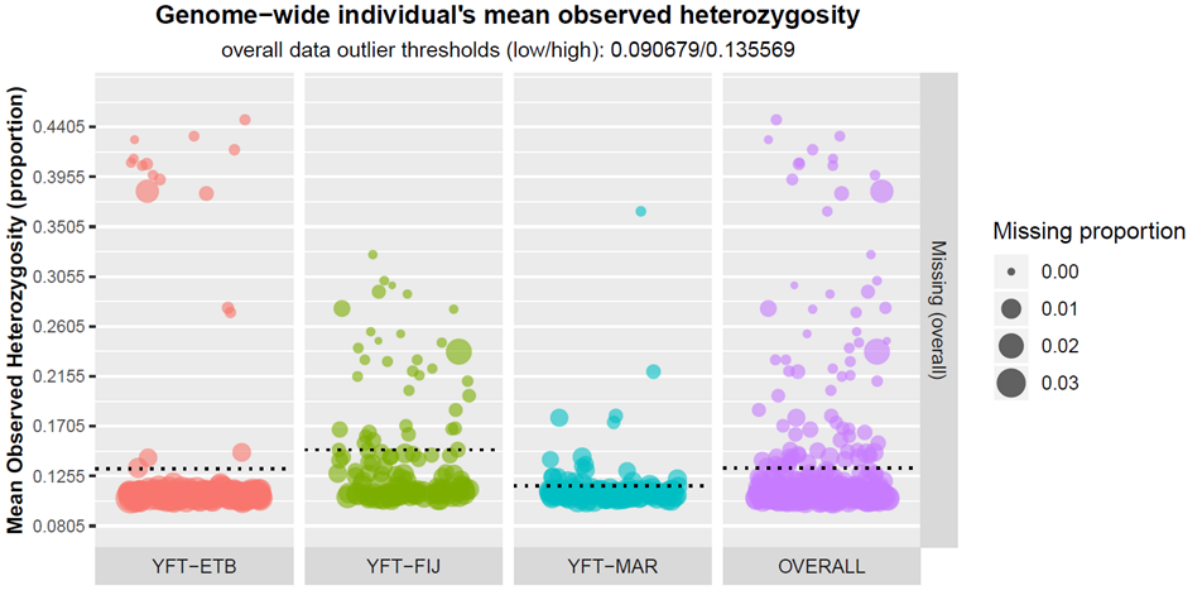
Samples: QC

Species	Region/EEZ	Year	Number samples received	Number genotyped (incl. replicates)	Number removed by step-wise QC (incl. replicates)
Albacore	Australia	2009	50	73	32
		2010	50	50	6
Albacore	New Caledonia	2013	25	36	9
		2014	37	44	7
		2016	45	64	40
Albacore	New Zealand	2008	47	63	25
		2010	47	47	7
Yellowfin	Australia	2006	52	65	32
		2013	50	77	33
Yellowfin	Fiji	2014	62	77	44
		2015	60	39	19
Yellowfin	Marshall Islands	2014	63	51	5
		2015	52	60	14
Bigeye	Australia	2017	50	66	
		2018	50	74	
Bigeye	Marshall Islands	2014	50	50	
		2015	50	50	
Bigeye	Solomon Islands	2013	72	96	
		2014	49	56	

Heterozygosity: albacore



Heterozygosity: yellowfin



Samples: QC

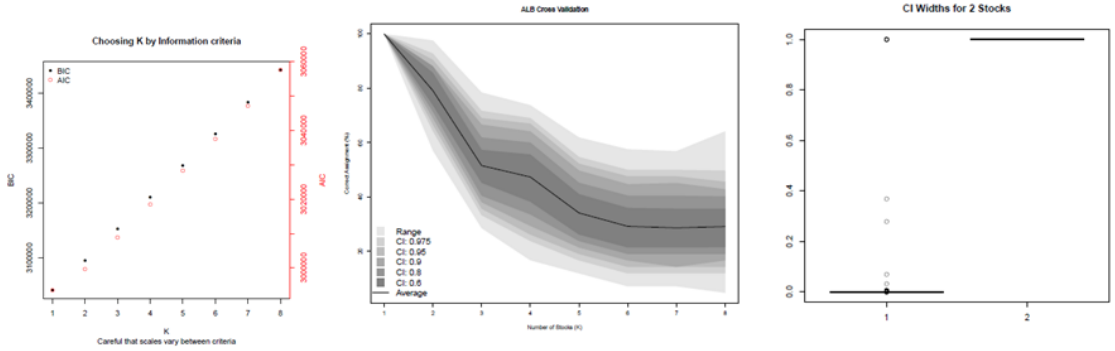
*9 identified as having insufficient DNA
^ 25 identified as having insufficient DNA
+ 13 identified as having insufficient DNA
# 2 identified as having insufficient DNA, 2 misidentified/mislabelled species

Species	Region/EEZ	Year	Missing data	Heterozygosity	Similar genotype
Albacore	Australia	2009	16	1	15 (all tech rep)
		2010	6		
Albacore	New Caledonia	2013		1	8 (all tech rep)
		2014			7 (all tech rep)
		2016		30	10 (all tech rep)
Albacore	New Zealand	2008	6	5	14 (12 tech rep)
		2010		5	2
Yellowfin	Australia	2006	8	15	9 (all tech rep)
		2013			33 (all tech rep/rep samples)
	Fiji	2014*		30	14 (13 tech rep)
		2015^	1	15	3 (2 tech rep)
Yellowfin	Marshall Islands	2014+		3	2 (1 tech rep)
		2015#		2	10 (9 tech rep)
Bigeye	Australia	2017			
		2018			
Bigeye	Marshall Islands	2014			
		2015			
Bigeye	Solomon Islands	2013			
		2014			

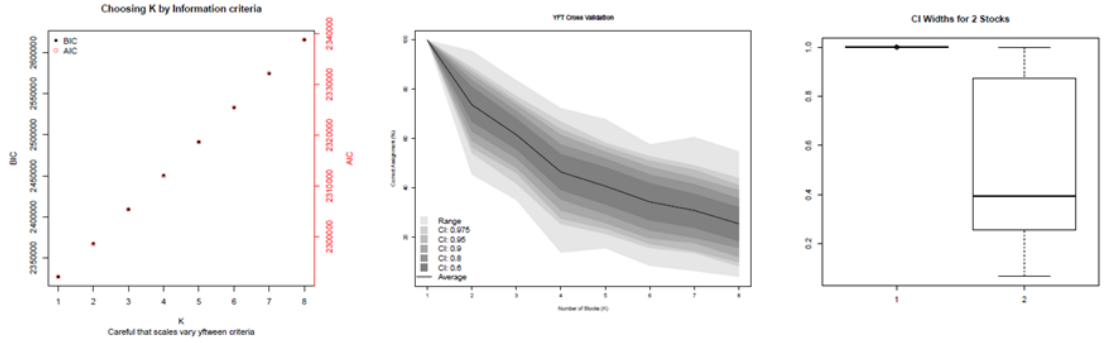
Mixture modelling

- is a mixture model not an admixture model
  - contemporary mixing, not evolutionary segregation (remember nature of samples)
- assumes that each samples belongs to one of K stocks
- purpose of the analysis is twofold
  1. to choose K and,
  2. to assign each sample to one of the stocks
- three approaches
  1. information criteria – value of K that minimises IC
  2. cross validation – value of K above which predictive performance declines
  3. bootstrapped confidence intervals – certainty associated with stock assignment

Model assignment: albacore



Model assignment: yellowfin



Interpretation of results

Data cannot confirm the presence of more than one genetic stock (breeding population) present in samples from ETBF and the two other sites investigated for each species

*these results only apply to the three sites included and cannot be extrapolated across the WPO region

Things to remember:
- samples consist of fish of multiple ages
- samples consist of multiple years
- samples collected across multiple months within a year
- samples do not represent spawning populations

In an ideal world* you would sample fish from multiple locations at the same time and sample fish that are spawning IF you are interested in establishing stocks associated with spawning rather than fish that are mixing on fishing grounds

* an ideal world rarely exists and is usually super expensive ($$, time, resources) to create

Interpretation of results

Without the WCPFC Tissue Bank and CSIRO historical samples this project would not exist

- however, users need to be aware:
  - what questions can be asked of samples
    - mixed individuals caught by fisheries
  - the limitations of the samples
    - varying quality of DNA
    - varying heterozygosity

    } cross-contamination, misidentification, mislabelling
  - the risks associated without due diligence to the above
    - misinterpretation of results

Next steps

Full preliminary results from tuna species for presentation to WCPFC-SC as an IP (late July)
- Include guidance for use of WCPFC Tropical Tuna Tissue Bank samples for genetic studies
- Recommendations for stock structure studies

Sequencing of striped marlin samples (NZ, Hawaii)

Sequencing of small numbers of NZ swordfish

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Thank you

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