Australian Government

Australian Centre for International Agricultural Research

Final report

Project full title

Foundation science and technologies to transform Indo-Pacific tuna assessment and management under climate change

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Fisheries Program on tuna research in the Pacific. In particular, it draws on the development and application of new genetic approaches to estimating abundance, connectivity, population structure and epigenetic age pioneered by the CSIRO team over the past decade (e.g. Bravington et al 2016 a&b, Bradford et al 2016, Grewe et al 2016; Preece et al 2015, Preece et al 2020; Mayne et al 2020; Hillary et al 2019, 2020; Davies et al., 2020, 2021) and the partnership developed with SPC (e.g. Anon 2023), the Indian Ocean Tuna Commission (IOTC) (Davies et al., 2018, 2020; Bravington et al 2018; Hillary et al 2022] and Indonesia (e.g. Proctor et al 2018,; Hoshino et al 2020; Davies et al., 2020; Lewis and Davies 2021) to build the capability to apply these methods to regional tropical tuna fisheries.

The tools and methods developed by the CSIRO team (sampling tools, workflows, data systems and statistical methods for epigentic age estimation and for abundance estimation using close-kin mark recapture and gene-tagging) make large-scale sampling, genotyping and analysis cost-effective for monitoring and management of large international fisheries, as demonstrated through their award winning application to southern bluefin tuna[1].

This project aims to accelerate the development and application of these transformational fisheries monitoring and management methods by completing a combination of necessary foundational science: i) epigenetic age calibration and high resolution genome sequencing; and ii) preparatory capacity building, including developing sampling procedures for regional observer programs and reviewing genetic processing capacity.

[1] *In 2018 The CSIRO SBT Team were awarded the CSIRO Award for Impact through Science and the Sir Ian McLennan Award for Industry for new genomic assessment methods and a scientifically tested management system for southern bluefin tuna that improved economic returns, stock status and community benefits.*

4 Objectives

1. Calibrate epigenetic age estimation models for yellowfin (YFT), bigeye (BET) and, if possible, skipjack[2] (SKJ) tuna to allow catch-at-age information to be estimated from routine tissue sample collection by observers and port sampling.
2. Complete high resolution genome sequencing and "re-sequencing" for YFT, BET and SKJ tuna to provide the genetic foundation to "map" specific markers on the genome for identification of kin (for abundance estimation), population structure (for provenance and connectivity) and adaptive loci (for assessing adaptive capacity).
3. Complete tissue sampling and handling experiments to refine Standard Operating Procedures (SOPs) that eliminate/minimise contamination and provide DNA extractions of consistent quality for future epigenetic ageing, close-kin mark recapture and population connectivity from routine large-scale tissue port/observer sampling of tropical tuna fisheries.
4. Review the human resources and infrastructure for routine genetic diagnostics (i.e., DNA extraction and dispatch, PCR facilities) across key Pacific Island countries as part of assessing the logistics and feasibility of large-scale tissue and/or DNA handling protocols.

[2] *Note, the calibration stage for epigentic age estimation requires a sample of "known" age individuals. In the case of YFT and BET these can be obtained from existing data sets of individuals that have been aged from reading of otoliths using validated methods. At present, such a data set does not exist for SKJ, however, ACIAR 2016-166 includes collection of samples and testing of approaches to age SKJ. If successful, this related project could provide the necessary "known" age data set for calibration of SKJ.*

5 Methodology

5.1.1 Calibration of epigenetic ageing for tropical tunas

This activity involved the calibration of epigenetic age prediction model for the three primary tropical tuna target species. In the case of YFT and BET, this was done using tissue samples from “known age” individuals, where the age has been estimated from reading of otoliths of the same individuals as per Mayne et al. (2021). Samples were sourced from existing CSIRO and SPC data holdings that have high quality age estimates available from previous projects (e.g. Farley et al 2020).

Calibration for SKJ tuna was more complicated in that there is currently no validated method for estimating the age of SKJ using hard-parts, such as otoliths and/or spines. This was an objective of FIS 2016-166, which has collected systematic monthly samples at four locations across the Indonesian archipelago. This provides one of the most comprehensive data sets available to address the age estimation problem for SKJ. Unfortunately, institutional disruption of the processing of these samples meant that calibration for SKJ was not able to be completed (See Davies et al 2023). This will be pursued in the future.

Otolith ages

Age estimates for BET and YFT were obtained directly from the study by Farley et al. (2020), who counted increments in sectioned otoliths to estimate age in decimal years. The fish were caught between 2010 and 2018 in the western and central Pacific Ocean. A muscle tissue sample for epigenetic age estimation was obtained for each aged fish from the PMSB (https://www.spc.int/ofp/PacificSpecimenBank). A small sub-sample was taken from the inside of each larger tissue sample following strict protocols to avoid potential cross-contamination.

Biomarker discovery

Previous studies have used conserved and age-associated CpG sites in other species to develop epigenetic clocks for their species of interest [20, 23, 24]. In study, we used CpG sites found to be age-associated in zebrafish (*Danio rerio*) and identified which ones were conserved in the tuna species analysed in this study [25]. Briefly, these CpG sites in zebrafish were identified as being age-associated by performing reduced representation bisulfite sequencing on known age zebrafish. In total, 1,311 CpG sites were identified as being age-associated (Pearson correlation, p-value < 0.01). Genomic conservation of the CpG sites was tested between the zebrafish (danRer10) and southern bluefin tuna (fThuMac1.1) genome using LASTZ with the following options: *--notransition --step=20 –nogapped* [26].

Primer design

Primer design was done using PrimerSuite for a multiplex PCR with bisulfite treated DNA [27]. This is the most cost-effective method to measure DNA methylation at multiple sites for a novel species compared to mammals where the array-based system is available. Primer design was carried out on the 48 most significantly age-associated CpG sites in tuna since this is the maximum number of primers that can be fit into one multiplex PCR design. Although multiple pools of primers can be developed, this would increase cost and require more DNA, which may not be available. Primer design was carried out with the following options: annealing temperature of 55°C, one pool of primers, unmethylated cytosines in the primer sequences, and two fusion sequences for secondary PCR (gacatggttctaca and cagagacttggtct).

DNA extraction, bisulfite treatment, multiplex PCR, and library preparation

DNA extraction was carried out using the DNeasy Blood & Tissue Kit (Qiagen, Cat. 69504) following the manufacturer’s protocol. Bisulfite treatment was carried out with

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Table 1. Tissue sampling treatments, procedures, and storage methods trialled on bigeye tuna captured during CP14.

Treatment		Replicate	*n*	Skin preparation	Sampling tool	Sampling tool cleaning	Storage	Additional procedures
T1	Current practices	T1.1	30	None	Knife	None	Freeze at ≤−4°C, sterile plastic bag	~50% of samples exposed to additional acts e.g. dropped on deck, contact another fish
		T1.2	30					
T2	Knife—dry wipe, soap, freeze	T2.1	30	Wipe with single-use dry cloth	Knife	Warm soapy water	Freeze at ≤−4°C, sterile plastic bag	None
		T2.2	30					
T3	Knife—dry wipe, soap, RNA*later*	T3.1	30	Wipe with single-use dry cloth	Knife	Warm soapy water	RNA*later* at ≤−4°C, sterile 5-ml vial	None
		T3.2	30					
T4	Knife—dry wipe, bleach, freeze	T4.1	30∗	Wipe with single-use dry cloth	Knife	3% bleach solution	Freeze at ≤−4°C, sterile plastic bag	None
		T4.2	30					
T5	Biopsy punch—dry wipe, RNA*later*	T5.1	30	Wipe with single-use dry cloth	Single-use, sterile biopsy punch tool	None	RNA*later* at ≤−4°C, sterile 5-ml vial	None
		T5.2	30					
T6	Biopsy punch—bleach wipe, RNA*later*	T6.1	30	Wipe with cloth soaked in 3% bleach solution	Single-use, sterile biopsy punch tool	None	RNA*later* at ≤−4°C, sterile 5-ml vial	None
		T6.2	30					
T7	Knife—no cleaning, freeze, subsample interior	T7.1	30	None	Knife	None	Freeze at ≤−4°C, sterile plastic bag	Subsample 1 cm^3 of interior tissue in laboratory

∗ One sample from T4.1 failed to sequence (see Results), leaving 389 samples available for further analysis.

Sub-sampling and laboratory preparation

In order to isolate potential cross-contamination events due to handling processes in the laboratory as part of the preparation for sending samples to the sequencing facility, samples from each treatment were processed in a different sequence at each step of the subsampling and plating process (i.e. samples from a single catch and processing event were not processed sequentially). In this way, cross-contamination due to "lab effects" would most likely be seen between samples processed sequentially and "sequencing effects" most likely between adjacent samples in the same plate.

Sequencing and genotype filtering

Tissues were sent to Diversity Arrays Technologies (DArT) in Canberra, Australia, for DNA extraction and sequencing. All samples were successfully sequenced with the exception of one, which was excluded from further analysis (See Anderson et al 2023, for full details and links to raw data files). The genotypes assigned by DArT were filtered for quality control prior to subsequent analysis using the following criteria: highest call rate SNP locus per contig, call rate (100%), read depth (>10×), *H*o (<50%), minor allele frequency (>5%), compliance with Hardy Weinberg Equilibrium (HWE) at $p > 0.0001$, and linkage equilibrium (<60%). Tests for HWE and linkage disequilibrium (LD) were conducted with PLINK 1.9 (Chang *et al.*, 2015) using the commands "—hardy" and "—*r*2," respectively.

Quantifying effects of sampling treatments on cross-contamination

The effects of the treaments were investigated by examining the level of variation in heterozygosity at two levels: i) replicate level; and ii) the individual fish level using a combination of linear and non-linear mixed effects models, with replicate as a fixed effect and individual fish as a random effect to account for the repeated measures nature of the multiple samples from individual fish (see Anderson et al. 2023).

Mitigating the impact of contamination

Samples were identified as outliers, and likely cross-contaminated, if they had a H_0 score greater than three times the standard deviation calculated for each individual fish. Of those identified as cross-contaminated, 24 were processed in the laboratory using the

subsampling procedure for treatment 7 (Table 2) that involved dissecting a small 1 cm^3 sample from within a larger (5 cm^3) block of tissue, to determine the extent this procedure could mitigate prior contamination (i.e. assuming the contamination is restricted to the surface layer and has not penetrated deeper into the block of tissue).

5.1.4 Review of current genetic capacity in the Pacific Islands

The current genetic infrastructure and human resources across key tropical tuna countries were reviewed from available documentation and consultations within SPC, selected in-country Health Departments and the Pacific Pathology Training Centre. The results of this review were used to make an initial assessment of the current capacity and future requirements for implementation of large-scale tissue sampling and processing.

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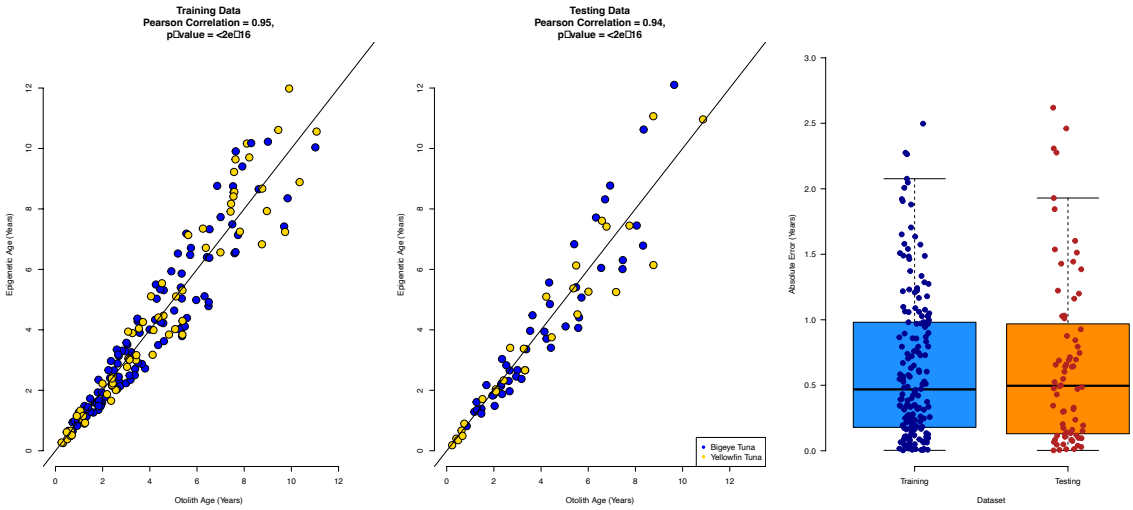
Training Data
Pearson Correlation = 0.95,
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Testing Data
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Epigenetic Age (Years)
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Bigeye Tuna
Yellowfin Tuna
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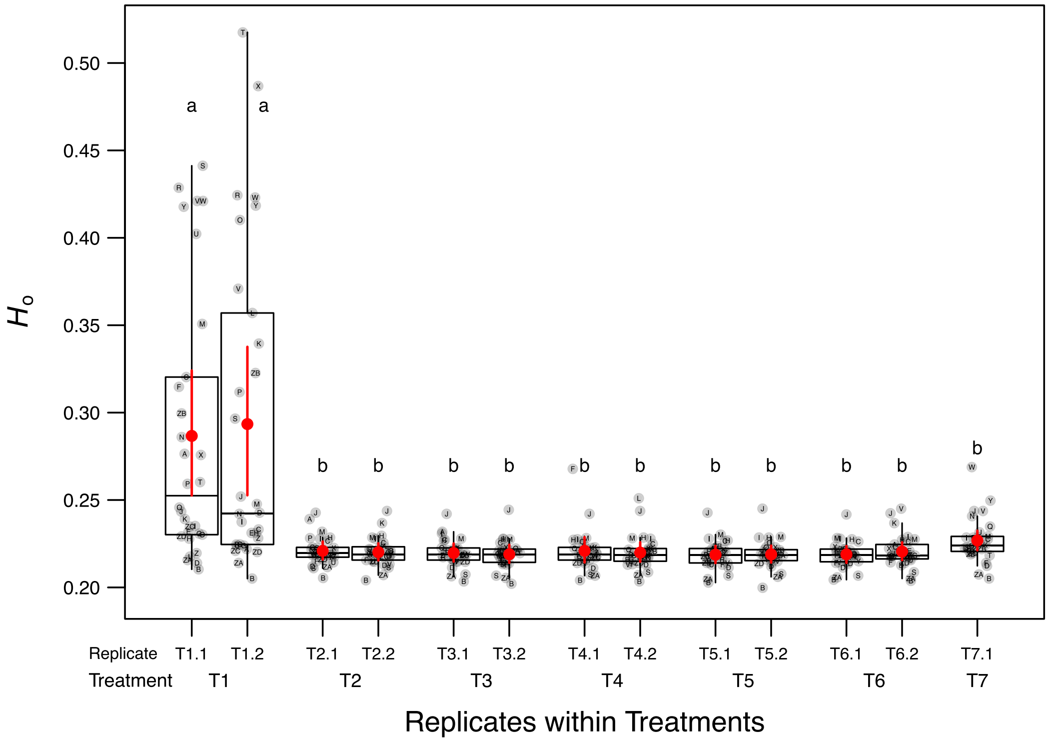

0.50
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Replicates within Treatments

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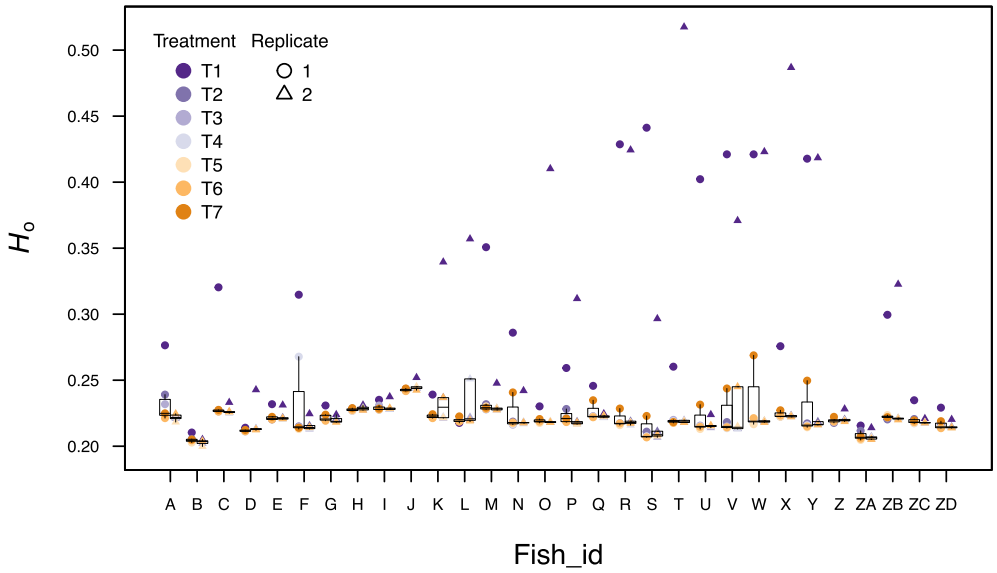
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Table 1). Similarly, these four countries plus the Federated States of Micronesia and the Marshall Islands land over 85% of the BET and YFT long-line catch (Figure 4, Table 4).

The concentration of longline landings in these six countries/federated states indicates that a substantial proportion of sampling of adults (and potentially DNA extraction) could be achieved through dedicated port sampling systems in each of these major countries. Further detailed examination of catch data (effort distribution, flag state, etc), complemented with information on population structure and distribution and landing of juveniles will be done as part of more detailed statistical designs for CKMR for each species (e.g Bravington et al 2021).

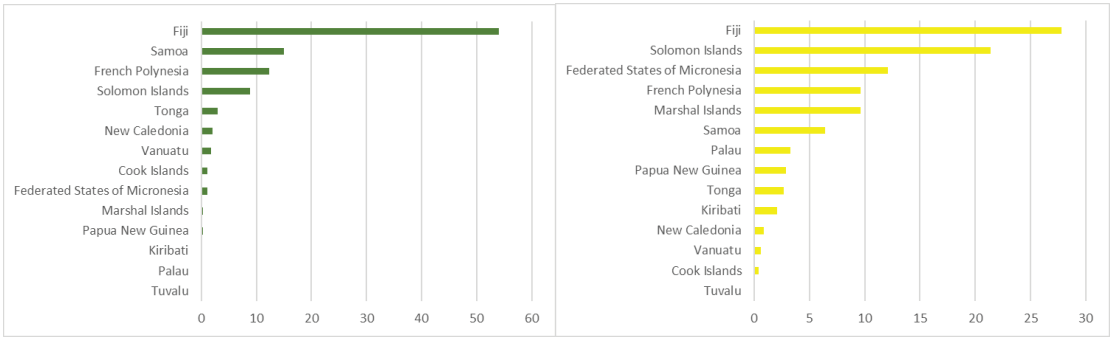

Figure 4. Proportions of albacore (left panel) and YFT and BET (right panel) unloaded in Pacific Island ports.

7.4.2 Container laboratories

In response to the COVID-19 pandemic, container laboratory systems have been delivered to the Pacific region to allow for in-situ COVID diagnosis. This initiative has been delivered through the Pacific Pathology Training Centre (PPTC) and Boxman Containers with donor support from the New Zealand Ministry of Foreign Affairs and Trade. The floor plan for these containers is prepared by PPTC and in-country Ministry of Health staff. The PPTC ensures that all the specifications of the container laboratory are met and that the units are fit for purpose and suitable for the Pacific Island setting.

Supply chains to the Pacific Island countries can be challenging, particularly for dangerous or “special transport needs” goods where only low volumes are required. To overcome these issues during the COVID pandemic, the containers were typically supplied with all equipment, reagents and consumables before shipping. On-going supply of reagents and consumables post COVID will need evaluation. Container Laboratory Systems have to date been delivered to: Tokelau, Niue, Kiribati and Fiji (Nadi and Labasa).

7.4.3 Molecular testing

Direct sequencing and microarray analysis have become standard tools for detecting a wide variety of organisms from clinical specimens. Closed molecular testing platforms such as GeneXpert and Abbott ID Now have applications for TB, HIV viral loads, Hepatitis, Chlamydia, Gonorrhoea, Multi Drug-resistant pathogens, RSV and Coronavirus. These are the most common use of this methodology in the Pacific region.

The adoption of molecular platforms is expected to increase with the introduction of ELISA and immunoassay analysers for confirmatory testing of disease. Real-Time PCR platforms are being introduced in Fiji, Marshall Islands, Nauru, Solomon Islands, Tonga, Palau and Tuvalu. Current PCR equipment is provided in Table 5.

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team at SPC. This will have a direct impact on the adoption and implementation of these new methods (e.g. McDonald et al 2023[3]; Mayne et al 2023c).

8.2 Capacity impacts – now and in 5 years

Review of genetics capability

The project has had direct impact on the scientific and technical capacity for fisheries monitoring and science through the collaboration with SPC and direct technology transfer beyond the project team. This and related projects (e.g. Bravington et al 2021, Anderson 2023, Anon., 2023) have substantially increased the understanding of the potential of these genetic methods for monitoring and assessment of Indo-Pacific tuna, the priority capacity building issues that need to be addressed for them to be implemented and secured significant internal and external funding to address these capacity needs. The SPC-CSIRO CCFSPF partnership agreement is specifically designed to build the scientific, logistic and technical capacity required to apply these methods to Pacific tuna.

8.3 Community impacts – now and in 5 years

The community impacts of this project in isolation are likely to be limited. However, in combination with related work underway for tuna and billfish in the Indo-Pacific, the impact has the potential to be substantial. This project provides some of the under-pinning science foundations for that broader work program to apply molecular methods to the monitoring and management of these globally significant fisheries and the communities that depend on them for food, livelihoods and national income.

8.3.1 Economic impacts

Improvements in the understanding of stock structure and connectivity and improved estimates of abundance for these globally significant stocks should reduce the risk of overfishing and improve the economic returns/reduce opportunity costs.

8.3.2 Social impacts

Potential to contribute to training and employment opportunities in Pacific Island countries the future should larger-scale roll out of genomics methods for fisheries management proceed.

8.3.3 Environmental impacts

Improvements in the understanding of stock structure and connectivity and improved estimates of abundance for these globally significant stocks should reduce the risk of overfishing.

8.4 Communication and dissemination activities

Peer review publication (Anderson et al, 2023)

Desemmination of methods among Indo-Pacifiic deepwater snapper fisheries science and management community through workshop participation.

[3] Four day capacity building workshop focussed on state of the art age determination methods for Indo-Pacific fisheries scientists. It included epigenetic ageing and the work presented in Mayne et al., 2023c for deepwater snappers.

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