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Population genetics and connectivity of albacore tuna in the Western and Central Pacific Ocean to inform sampling design and marker development for close-kin mark-recapture (CKMR)

WCPFC-SC21-2025/SA-WP-10

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Executive Summary

At SC20, SA-IP-04 reported on population structure of albacore tuna (*Thunnus alalunga*) in the South Pacific (Macdonald et al., 2024). Results indicated sufficient differences in genetic and otolith metrics that were indicative of population structure to warrant further investigation. This working paper reports on the recommendations of SC20 to expand the spatial sampling to better understand genetic population structure of South Pacific albacore tuna (SPA) from a wider distribution of locations south of the equator within the WCPO. Analyses of these additional samples provides evidence of at least three distinct genetic groups of SPA. While individuals from all three of the identified genetic groups were present in most of the sampled locations, there was a clear spatial distribution that indicated genetic differentiation of SPA among sampling locations within the WCPO.

The current Close-Kin Mark-Recapture (CKMR) sampling program provides adequate coverage of the South Pacific WCPO region sufficient to support unbiased estimates of population parameters (spawner abundance, mortality, relative reproductive output) as well as estimates of generational connectivity (i.e., siblings, half-siblings and parent-offspring pairs). The CKMR sampling program alone, however, is insufficient to fully characterise the geographical location and distribution of specific spawning activity related to the three identified genetic groups across the WCPO. A more complete understanding will require targeted sampling of actively spawning adults and/or larval collection in currently unsampled areas to confirm spawning locations and assign individuals to their genetic group with high confidence. Continued CKMR sampling will contribute to monitoring the status of these genetic spawning components, using genetic markers to reliably identify group membership. Understanding this information will ultimately impact on structuring and generating the final CKMR population model. These findings from the WCPO will also highlight the need to investigate population structure and connectivity across the broader Pacific region.

These initial findings: i) support the efficacy of the current sampling design and program established as part the CKMR feasibility study; ii) identified the presence of at least 3 distinct genetic groups of South Pacific albacore in the WCPFC Convention Area (WCPFC-CA); and, iii) identified the need to consider assessment approaches and potential management measures that take into account the mixed genetic composition of the fishery demonstrated by these results.

Recommendations:

- The established sampling program for CKMR be maintained to provide samples required for future monitoring and assessment of SPA and, potentially, tropical tuna species.
- Additional sampling of SPA is required in the EEZs of Solomon Islands, American Samoa, and Tuvalu, as well as current gaps in coverage between the east coast of New Zealand and 165°W at a latitudinal band ~40°S to confirm the total number of genetic groups and their spatial distribution in the WCPO.
- Sampling is also required further east of 145°W to confirm the presence/absence of additional genetic groups of SPA east of the WCPFC-CA.
- CKMR is recommended to be an effective approach to providing information to support the implementation of future stock assessment and potential management requirements for SPA.

Background

Understanding population structure and connectivity of a species is an important foundation for the design and implementation of unbiased estimation of abundance and other population parameters using Close-Kin Mark-Recapture (CKMR) (Bravington et al. 2016 a, b; Davies et al.

2014). The use of genetic relatedness data coupled with spatial modelling augment absolute abundance and connectivity of CKMR studies and in turn can help inform assessment models (Tremblay-Boyer et al. 2024). Unidentified population structure within a CKMR study range can bias the resulting estimates. Thus, the presence of population structure should be incorporated both in sampling design and in spatial models, such that sampling efforts are effectively distributed to sample all population subgroups of interest, and the results are applied to an appropriate stock area.

The 2021 South Pacific albacore (*Thunnus alalunga*; SPA) stock assessment presented to SC17 (Castillo Jordán et al. 2021) raised both of these topics. It acknowledged that:

"The most influential uncertainty of those considered in this assessment was the assumption related to movement of fish among the model regions. Further research on albacore movement and population mixing across the entire South Pacific should be a priority. Given the difficulty of tagging albacore, genetic and otolith-based approaches are recommended."

It also noted:

"… the development of the Close Kin Mark Recapture (CKMR) methods that can provide information on population scale and stock structure, along with other fishery-independent information on uncertain biological processes, and we strongly recommend that this approach is considered for South Pacific albacore …"

In response to these recommendations, a two-phase study was implemented to better quantify population structure in SPA in the WCPO. Phase-1 used otolith shape and genetic data collected in a very specific sampling design, the results of which were presented at SC20 (Macdonald et al. 2024). Both genetic and otolith markers supported the presence of reduced connectivity of SPA between the western and easternmost regions of the WCPFC Convention Area (WCPFC-CA). This result is consistent with the published literature, with similar conclusions made by studies exploring genetic markers (e.g. Takagi et al. 2001; Montes et al. 2012; Anderson et al. 2019, but see Laconcha et al. 2015), otolith microchemistry (Macdonald et al. 2013), growth variability (Williams et al. 2012; Farley et al. 2021) and gonad development (Farley et al. 2013). However, most empirical studies to date have been constrained by a lack of spatial and temporal resolution and/or structured sampling at the scale of the WCPFC convention area.

Based on the Phase-1 results, a more expansive sampling program was undertaken as part of the Climate Science for Ensuring Pacific Tuna Access (CSEPTA) and WCPFC project. Phase-2 focused solely on genetic markers due to the consistency of results from both otolith and genetic analysis in Phase-1. Samples from multiple sampling locations were collected to gain insight regarding both longitudinal and latitudinal variation at a wider spatial scale pattern among adult and juvenile fisheries. Where possible, additional sites were chosen and archived samples were used to investigate temporal variability of SPA population structure within the WFCPC-CA.

This Working Paper reports key results from the Phase-2 that will inform the refinement and extension of sampling strategies for CKMR abundance estimation project currently underway. This information will help better reflect the underlying population structure and connectivity within the stock assessment models for SPA ahead of the 2027 assessment.

Methods

Sample collection

Samples used for analysis in this report were collected over a 16-year period from 2008 through to 2023 through different CSIRO and SPC research projects. This included SPA subsampled

from the WCPFC Tissue Bank and dedicated sampling under the CSEPTA project. The samples come from an area bounded by Australia in the west through to French Polynesia in the east, with large fish caught by longline fleets operating in northern latitudes (12ºS to 25ºS), where spawning occurs (Farley et al., 2013), as well as smaller juvenile fish sampled from the troll fisheries in the southern latitudes (30 º to 45 ºS) (Figure 1 and Table 1). Muscle tissue samples were collected using single-use biopsy tips (Bradford et al., 2016). Immediately after collection, the samples were chilled at 4°C for approximately 1 hour and then transferred to –20°C for longer term storage and shipment to the CSIRO Marine Laboratories in Hobart.

Table 1. Sampling locations by area: TAS (Tasmania, Australia); COR (South Coral Sea); NC (Noumea, New Caledonia); NZ (Westport, New Zealand); FIJ (Suva, Fiji); TON (Nuku'alofa, Tonga); TOK and TAU (combined in analyses from boats operating in overlapping waters off Tokelau and Te Tautua, northern Cook Islands, respectively); FP (Papeete, French Polynesia); and USF (US flagged high seas fishing vessel). Bolded years indicate catch sampling year, N (number of fish sampled), average length of sample set (fork length, FL cm), and range of lengths (FL cm) for South Pacific albacore tuna used in the current study. See Appendix 1 for additional sampling locations for each coded region.

Area Code	2008	2010	2013	2014	2016	2017	2018	2022	2023	2024	N	Avg length (cm)	Length range (cm)
TAS						17	40				57	45.2	43 - 47
COR							46				46	89.6	81 - 96
NC			27	37	40			108			212	94.6	78 - 106
NZ	46	45							941		1032	52.7	47 - 76
FIJ										144	144	94.5	84 - 103
TON										93	93	93.7	85 - 101
TOK										48	48	93.7	85 - 107
TAU										44	44	92.6	85 - 107
FP								91			91	93.8	84 - 105
USF									100		100	65.7	53 - 82
Total											1867		

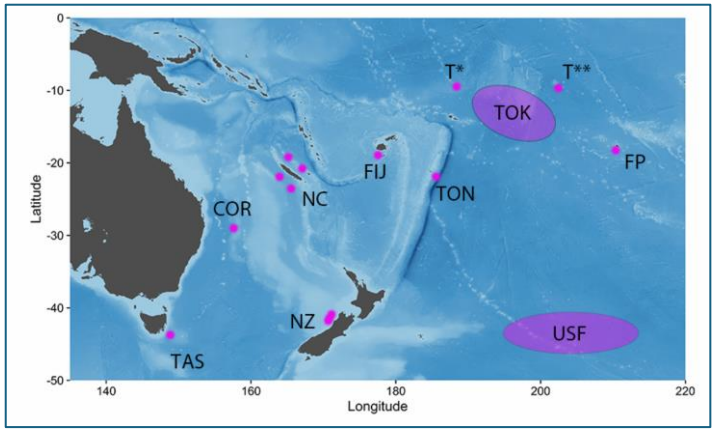

Figure 1. Approximate catch locations of South Pacific albacore tuna included in the current study as detailed in Table 1. Fish from Tokelau (T*) and Te Tautua (T**; northern Cook Islands) were combined into the area coded as TOK as they were caught on two boats fishing an overlapping region and appeared to be genetically indistinguishable from each other. The USF sampling area refers to fish caught by US flagged fishing vessels that caught albacore on the high seas fishing grounds (WCPFC area 1F) within the subtropical convergence zone east of New Zealand and south of French Polynesia. Other area codes refer to pooled samples from boats landing their catch at sampling ports as listed in Table 1.

DNA Extraction, and Quality Control and Genotyping

In the laboratory, muscle tissue biopsies were placed in 96 well plates and DNA was extracted using Qiagen QIAamp 96 DNA QIAcube HT Kit (Cat. No. 51331; Qiagen, Hilden, Germany) performed on a Hamilton Microlab STAR automated liquid handling platform (Detailed procedures as per description in the Appendix 2). Quality control (QC) testing was performed on 25% of the samples from each plate to evaluate DNA extraction efficiency—including DNA concentration, purity, and integrity—and to assess plate-level consistency. The resulting DNA extract was split into two plates, an archive plate retained at CSIRO and a DNA template plate. The template plates of DNA were submitted to Diversity Arrays Technologies (DArT) in Canberra where genotype sequencing was completed (see Appendix 2) to produce data used in downstream population analysis at CSIRO.

Population Genetic Analysis

The R-package RADIATOR (Gosselin et al. 2020) was used to filter out poor quality loci (i.e., Single Nucleotide Polymorphism (SNP) loci with low repeatability or low coverage), poor quality individuals (i.e., too much missing data due to poor DNA extraction), individuals exhibiting DNA cross-contamination (i.e., significantly higher than average heterozygosity), and removal of duplicated samples (i.e., technical replicates and double sampled fish). The final filtered dataset of 1297 SPA and 5202 loci were used for further downstream analysis. Discriminant Analysis of Principal Components (DAPC) from the *adegenet* pipeline (Jombart, 2008; Jombart et al., 2010) was used to examine genetic relationships among sampling locations. DAPC is a likelihood-based clustering algorithm that classifies individuals into the most logical groups based on comparisons of metrics of genetic variability within versus between chosen group levels (K). Inference of the number of genetically distinct groups is also possible within a dataset by comparing how confidently the algorithm can organise individuals into specified K genetic groups. In addition, analysis of pairwise F_{ST} (Weir and Cockerham, 1984) was performed to compute overall and pairwise F_{ST} values that were estimated with confidence intervals based on 2000 bootstraps of markers (resampling with replacement).

Results

Sample coverage

Between 44 and 1,032 individuals were collected from each of 10 sampling regions, for a total of 1867 individuals, providing representative coverage of much of the SPA fisheries (Table 1; Figure 1). Length data indicated that fish from the longline fishery in the northern regions were mostly adults aged 4+ years, and fish from the troll fisheries from the southern regions were mostly juveniles aged 1–3 years (Farley et al., 2014; 2021).

Temporal stability of genetic profiles by location

Analysis of historical DNA samples from previous projects permitted multiple year analysis (Table 1) that demonstrated temporally stable genetic patterns for three locations: Tasmania (2017, 2018), New Caledonia (2013, 2014, 2022) and New Zealand (2008, 2010, 2023). Given this temporal stability, the individuals from these locations were pooled for each location. Interestingly, the genetic signatures of the three New Caledonia tissue collections, remained unchanged over a 10-year period sampling period. For Tasmania, only two consecutive years of sampling could be compared, however, the same sized fish were sampled in each of 2017 and 2018 (43–47 cm FL, ~age 1 juveniles) indicating this was a comparison of two separate year classes that demonstrated a temporally stable genetic signal. Similarly, New Zealand juveniles sampled from Westport showed a clear stable genetic signal across almost 16 years (2008, 2014, and 2023).

Number of genetic groups

DAPC cluster analysis partitioned all fish into three clear and separate genetic clusters within the first PC1/PC2 (Figure 2). Analysis assuming K=2, K=4, and K=5 (figures not shown) resulted in very similar groupings: i.e. three distinct groups, indicating that the K=3 result was quite robust.

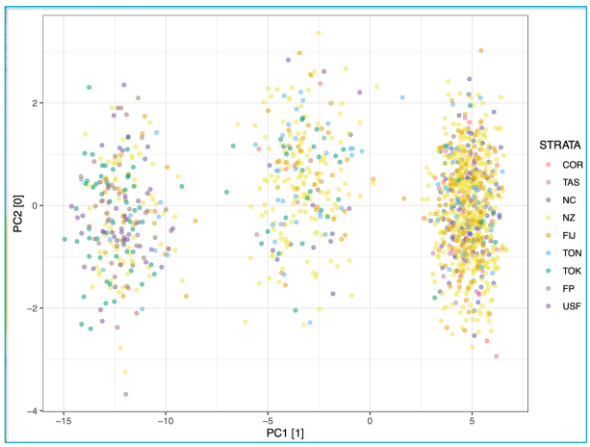

Figure 2. Genetic cluster plot produced from DAPC analysis at K=3 (also observed when K=4 and K=5) showing three well defined genetic clusters that explain >90% of variation among the samples. Dots represent individual fish colour coded by sample location. Note, individual fish from each genetic group are distributed unevenly across most locations.

A pairwise F_{ST} analysis was used to index the degree of relative genetic differentiation among paired location comparisons on a 0–1 scale, with lower values indicating greater genetic similarity and increased relatedness between compared populations (Figure 3). This matrix of comparisons echoes the results of the DAPC cluster plots. It supports a population structure of

at least three major genetic groups with the potential for additional partitioning within the WCPO (Figure 3).

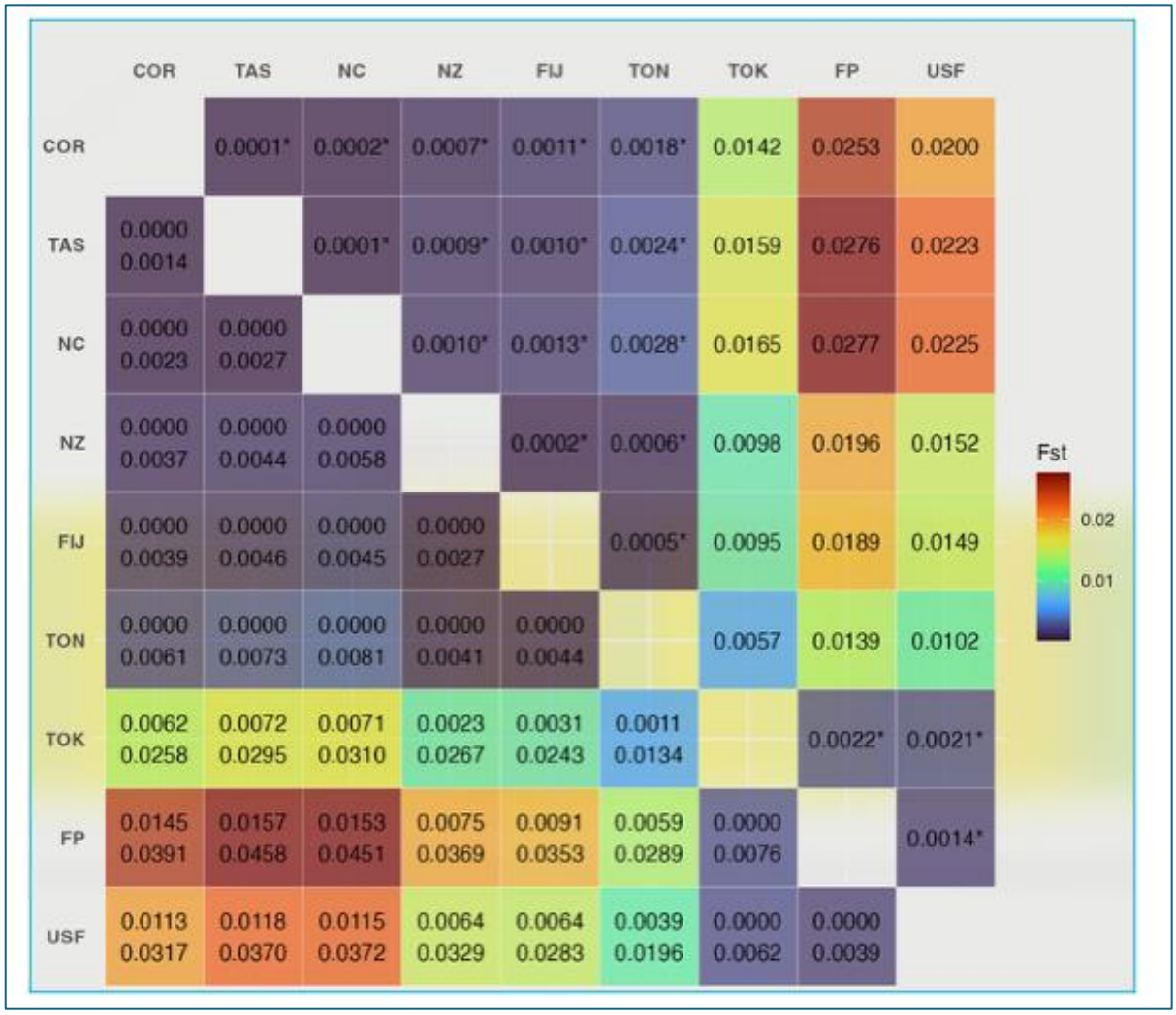

Figure 3. Matrix of F_{ST} pairwise comparisons with absolute F_{ST} values above the diagonal and confidence intervals for F_{ST} (minimum and maximum F_{ST}) below the diagonal. Larger values and warmer colours indicate greater relative genetic differentiation between compared sample location pairs. Colour scale from dark blue indicating relatively low genetic differentiation, to burnt orange at the maximum observed genetic similarities calculated between paired comparisons. For example, NC and FP are estimated to be very different (0.0277) while NC and TAS are estimated to be almost zero difference (0.0001).

Spatial distribution of genetic groups

The relative F_{ST} distances calculated between paired sample locations are consistent with adult samples from the northern spawning areas more closely matching genetic profiles of juveniles sampled from regions to the south (Figure 3). Additionally, observed DAPC probability plots for individual fish, which plot out as a solid colour, demonstrate a high probability (approaching 100%) of their assignment to one of the three specific K genetic groups (Figure 4, a). Thus, single cumulative average bar plots (Figure 4b) can be considered as representing the frequencies of each of the three genetic groups present within each of the sampled locations (Table 2). These

plots are consistent with north/south similarities calculated for relative F_{ST} comparisons and illustrate a pronounced geographical discontinuity between western most versus eastern most sampled locations (Figure 5, Table 2).

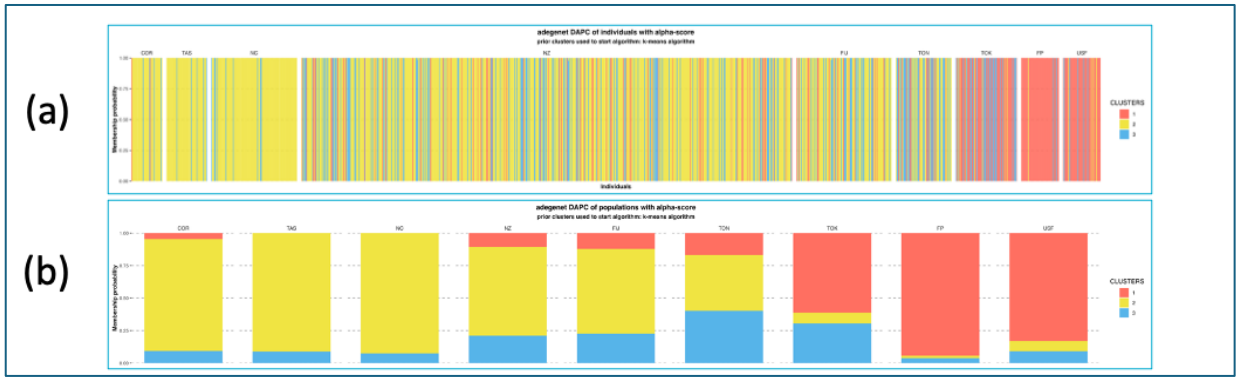

Figure 4. (a) DAPC barplots of individual fish indicating the probability of assignment of an individual to one of three K genetic groupings. The high probability of individual assignment is indicated by the solid colour of individual bars. (b) Average cumulative proportions of each of the three identified genetic groups in each sampling region.

Table 2. Proportion of each genetic group (1, 2, 3) observed at each sampling location.

Sample Location	Group 1	Group 2	Group 3
COR	0.04	0.85	0.11
TAS	0	0.90	0.10
NC	0	0.92	0.08
NZ	0.11	0.68	0.21
FIJ	0.12	0.65	0.23
TON	0.17	0.43	0.40
TOK	0.61	0.08	0.31
FP	0.94	0.02	0.04
USF	0.82	0.08	0.10

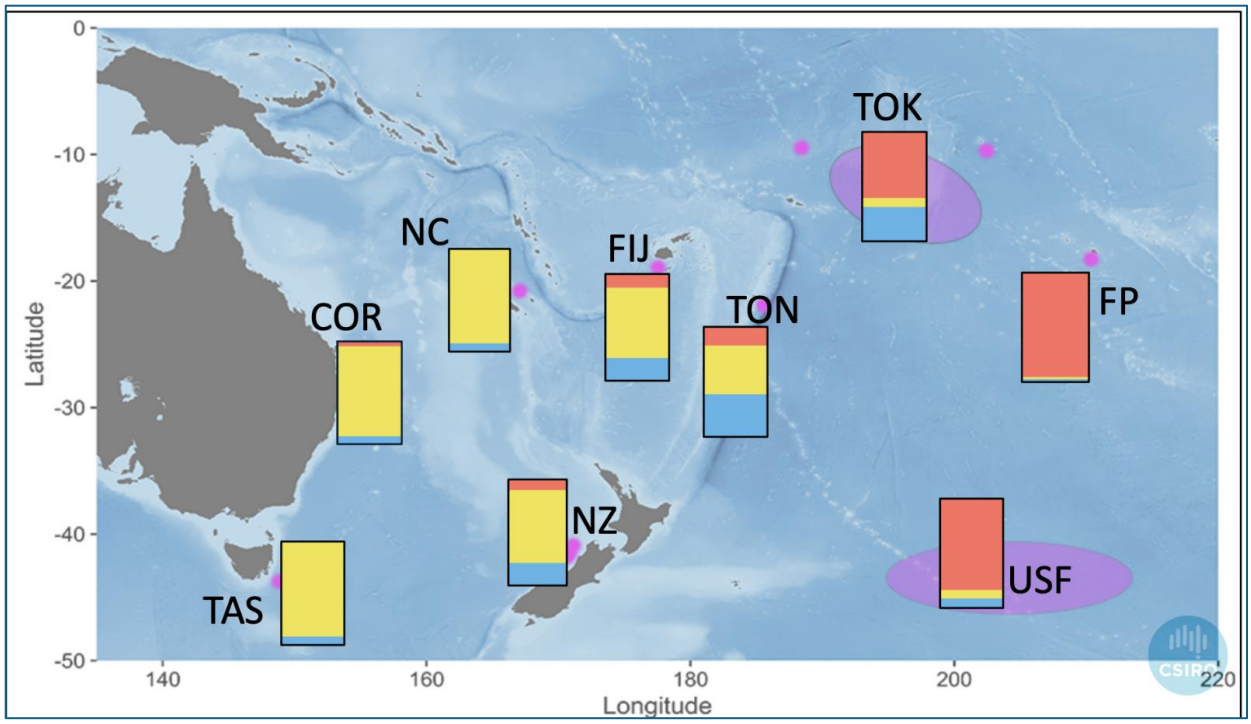

Figure 5. Geographic plot of cumulative DAPC plots indicating proportion of each of the three identified genetic groups for each sampling location. Each bar plot has been plotted at the approximate geographical catch location. Bar plot labels follow area codes listed in Table 1. For convenience, actual proportions values are reported in Table 2.

Discussion

Analysis of genetic markers (SNPs) presents strong evidence to refute the null hypothesis of a single panmictic population of SPA in the WCPO. The identification of three clearly separated groups in the DAPC cluster indicates restricted gene flow among a minimum of three genetically distinct groups. The clear separation observed between clusters is indicative of minimal to no genetic connectivity among the three putative populations. Individuals assigned to each of the three genetic groups were present in collections from each of the five adult sampling regions (i.e., NC, FIJ, TON, TOK and FP). However, the proportions of each of the three groups present within a sampling area are varied substantially and in a non-uniform manner (Table 2). This strongly suggests that the adult and juvenile fisheries for SPA in the WCPO are "mixed fisheries", i.e. harvesting different proportions of the three distinct genetic groups in different regions of the South Pacific.

Spatially and temporally stratified sampling of actively spawning adults and/or larvae is required to further delineate the spawning distributions of the three genetic groups, identify recruitment regions for each group, and determine if there are any additional genetic groups within the WCPFC-CA. This will also assist in testing hypotheses for the underlying mechanism(s) generating the regional genetic partitioning evident in these results. To help further resolve the origin and confirm the boundaries of the observed genetic groups, we recommend analysis of larvae and/or actively spawning adults sampled across the spawning region (see Farley et al 2013).

The genetic composition of sampled juvenile locations showed strong similarity to that of adults collected approximately northward of the juveniles. The genetic relationships observed among paired comparisons of adult and juvenile albacore sample locations provides strong

evidence of a primarily ~north-south latitudinal migration of juveniles. This is consistent with juvenile fish moving southward to reach productive feeding grounds of the sub-tropical convergence zone. These correlations, based on F_{ST} genetic distance, pair New Caledonia (adults) with Tasmania (juveniles), and Fiji (adults) with New Zealand (juveniles) and indicate that these two locations share the closest genetic adult-juvenile relationships with each other. A similar, albeit slightly weaker, north-south pattern also appears to be present between the French Polynesia adults and the juvenile samples collected by the US troll fleet in the Pacific gyre in the south. The slightly greater genetic distance observed between these "paired samples" and those of NC-TAS and FIJ-NZ could be due to a small, but limited number of western origin juveniles making additional longitudinal migrations. This hypothesis is consistent with available conventional tagging data, which indicates that while the majority of tagged juveniles move northward, a small number have made considerable longitudinal movements (Moore et al. 2020, Castillo Jordán et al. 2021). Additional targeted spatio-temporal sampling is required to investigate whether the north-south migration pattern in the genetic data is both spatially and temporally stable across the broader south Pacific.

Results from this study directly inform the efficacy of the sampling design being the current CKMR abundance estimation of SPA. They demonstrate a need to distribute sampling effort across the range of at least three genetically distinct groups of SPA. Importantly, consideration needs to be given to the degree of genetic separation indicated by results from the current study. The current results highlight the importance and indicate that in addition to the Tasman Sea juvenile grounds (west of New Zealand), regular (and substantial) sampling of fish from the US troll fleet will be required, as well as addressing the current sampling gap within the eastern NZ EEZ. While this eventuality was considered by Bravington et al. (2020; 2021), and Tremblay-Boyer et al. (2024), previous CKMR feasibility modelling considered that all juvenile sampling could be covered by sampling of SPA from the NZ troll fishery (Bravington et al. 2020; 2021; Tremblay-Boyer et al. 2024). This study indicated insufficient mixing of juveniles in the NZ fishery that potentially originate from French Polynesia to be confident that the original proposed CKMR design would provide sufficient coverage of the predominant (>90%) genetic group present in French Polynesia. However, the currently accepted CKMR design does in fact take this into account and provides a good CKMR sampling strategy that incorporates individuals representative of the three genetic groups.

In addition to supporting the evaluation and refinement of the sampling designs for CKMR, the results of this study have implications for the regional structure used in the stock assessment of SPA. The most recent assessment of SPA (Teears et al. 2024) assumed a single stock spanning 0° to 50°S and from 140°E to the west coast of South America (approximately 70°W), with regional subdivision consistent with the boundaries of the WCPFC-CA (inclusive of the overlap area) and the Inter American Tropical Tuna Commission (IATTC) Convention Area, and with no longitudinal breaks within the WCPFC-CA. The identification of three genetically distinct groups within the boundaries of the WCPFC-CA suggest that future assessments need to take this structure and limited connectivity within the WCPFC-CA into consideration. This will best be addressed by ongoing implementation of the CKMR sampling program to provide a regular source of the necessary samples for periodic analysis. As all three of the currently identified genetic groups are covered by the present CKMR sampling program, it will be possible to estimate spawning abundance, adult mortality and relative productive contribution for each of these genetic groups, given sufficient samples over sufficient years (see SC-WP-14, 2025). In addition, to these important productivity parameters, CKMR also allows connectivity within and between generation to be estimated through the identification of parent-offspring pairs, siblings and half-siblings pairs. This form of connectivity data is much more

informative, from a stock assessment and fisheries management perspective, than conventional population genetics (see Davies et al. 2014); and is also much more easily assimilated into integrated stock assessment models.

In conclusion, the current sampling program instituted as part of the feasibility study for CKMR provides the necessary coverage of the majority of the regions required to generate unbiased estimates of population parameters (spawner abundance, mortality, relative reproductive output) as well as estimates of generational connectivity. However, it is not sufficient, at present, to fully characterise the spatial distribution and connectivity of the three identified genetic groups across the entire WCPO region. The latter requires dedicated sampling of actively spawning individuals, and/or collection of larvae, in a number of regions not sampled in this study to provide conclusive evidence of spawning locations and genetic assignment of individuals to their genetic group. Ongoing sampling for the purposes of CKMR will provide the necessary information for monitoring the status of spawning components of these genetic groups, with the population markers providing the basis for high confidence assignment of individuals to their genetic group. These results for the WCPO suggest that a broader analysis of the Pacific wide structure and connectivity of albacore tuna is warranted. These findings: i) support the efficacy of the current sampling design and program established as part the CKMR feasibility study; ii) identify at least three distinct genetic groups of SPA in the WCPO convention area; and, iii) identify the need to consider assessment approaches and potential management measures that take into account the mixed genetic composition of the fishery demonstrated by these results.

Recommendations

- The established sampling program for CKMR be maintained to provide samples required for future monitoring and assessment of SPA and, potentially, tropical tuna species.
- Additional sampling of SPA is required in the EEZs of Solomon Islands, American Samoa, and Tuvalu, as well as current gaps in coverage between the east coast of New Zealand and 165°W at a latitudinal band ~40°S to confirm the total number of genetic groups and their spatial distribution in the WCPO.
- Sampling is also required further east of 145°W to confirm the presence/absence of additional genetic groups of SPA east of the WCPFC-CA.
- CKMR is recommended to be an effective approach to providing information to support the implementation of future stock assessment and potential management requirements for SPA.

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References

Anderson, G., Hampton, J., Smith, N., and Rico, C. 2019. Indications of strong adaptive population genetic structure in albacore tuna (*Thunnus alalunga*) in the southwest and central Pacific Ocean. Ecology and Evolution, 9: 10354–10364. 19

Bradford, Russell W., Hill Peta, Davies Campbell, Grewe Peter. 2016. A new tool in the toolbox for large-scale, high-throughput fisheries mark-recapture studies using genetic identification. *Marine and Freshwater Research* **67**, 1081-1089. https://doi.org/10.1071/MF14423

Davies, C.R., Bravington and Thomson, R. 2014. *Advice on Close-Kin Mark-Recapture for estimating abundance of eastern Atlantic bluefin tuna: a scoping study*. Final Report to the ATLANTIC-WIDE RESEARCH PROGRAMME ON BLUEFIN TUNA (ICCAT GBYP – PHASE 5) of the international Commission for the Conservation of Atlantic Tuna. 34p.

Bravington, M.V., Grewe, P.M., and Davies, C.R. 2016a. Absolute abundance of southern bluefin tuna estimates by close-kin mark-recapture. Nature Communications 7: 13162.

Bravington, M. V., Skaug, H. J., and Anderson, E. C. 2016b. Close-Kin Mark-Recapture. Statistical Science, 31(2):259 – 274

Bravington, M., Farley, J., Hampton, J., and Nicol, S. (2020). Preliminary analyses for a CloseKin Mark Recapture feasibility study in WCPO. Information paper to the Sixteenth RegularSession of the Scientific Committee of the Western Central Pacific Fisheries Commission,WCPFC-SC16-2020/SA-IP-15. Virtual meeting, 12–19 August 2020.

Bravington, M., Nicol, S., Anderson, G., Farley, J., Hampton, J., Jordan, C. C., and MacDonald, J. (2021). South Pacific Albacore Close-Kin Mark-Recapture: update on design. Information paper to the Seventeenth Regular Session of the Scientific Committee of the Western Central Pacific Fisheries Commission, WCPFC-SC17-2021/SA-IP-14. Virtual meeting, 11–19 August2021

Castillo Jordán, C., Hampton, J., Ducharme-Barth, N., Xu, H., Vidal, T., Williams, P., Scott, F., Pilling, G., and Hamer, P. 2021. Stock assessment of South Pacific albacore tuna. SC17/SA-WP-02. Seventeenth Regular Session of the Scientific Committee of the Western and Central Pacific Fisheries Commission. Online meeting, 11-19 August 2021. https://meetings.wcpfc.int/node/12551

Farley, J., Krusic-Golub, K., and Eveson, P. 2021. Updating age and growth parameters for South Pacific albacore (Project 106). SC17-SA-IP-10. Seventeenth Regular Session of the Scientific Committee of the Western and Central Pacific Fisheries Commission. Online meeting, 11-19 August 2021. https://meetings.wcpfc.int/node/12568

Farley, J. H., Williams, A. J., Hoyle, S. D., Davies, C. R., and Nicol, S. J. 2013. Reproductive dynamics and potential annual fecundity of South Pacific albacore tuna (*Thunnus alalunga*). PLoS One, 8(4), e60577.

Farley JH, Hoyle SP, Eveson JP, Williams AJ, Davies CR, Nicol SJ (2014) Maturity ogives for South Pacific albacore tuna (Thunnus alalunga) that account for spatial and seasonal variation in the distributions of mature and immature fish. PLoS ONE 9(1): e83017. doi:10.1371/journal.pone.0083017.

Gosselin, T., Lamothe, M., Devloo-Delva, F., Grewe, P. (2020). radiator: RADseq Data Exploration, Manipulation and Visualization using R. R package version 1.1.5 https://thierrygosselin.github.io/radiator/. doi: 10.5281/zenodo.3687060

Jombart, T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics, 24: 1403–1405.

Jombart, T., Devillard, S. & Balloux, F. (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet* **11**, 94 (2010). https://doi.org/10.1186/1471-2156-11-94

Laconcha, U., Iriondo, M., Arrizabalaga, H., Manzano, C., Markaide, P., Montes, I., Zarraonaindia, I., et al. 2015. New nuclear SNP markers unravel the genetic structure and effective population size of albacore tuna (*Thunnus alalunga*). PLoS ONE, 10(6): e0128247.

Macdonald, J. I., Farley, J. H., Clear, N. P., Williams, A. J., Carter, T. I., Davies, C. R., and Nicol, S. J. 2013. Insights into mixing and movement of South Pacific albacore *Thunnus alalunga* derived from trace elements in otoliths. Fisheries Research, 148: 56–63.

Macdonald, J., Anderson, G., Krusic-Golub, K., Prioul, F., Cuewapuru, C., Hosken, M., Barthelemy, V., Raoulx, T., Grewe, P., Nicol, S., and Panapa, A. 2024. Spatial Structure and Regional Connectivity of South Pacific Albacore Tuna in the WCPO and EPO. Information paper to the Twentieth Regular Session of the Scientific Committee of the Western and Central Pacific Fisheries Commission, WCPFC-SC20/SA-IP-04. Manila, Philippines, 14-21 August 2024. https://meetings.wcpfc.int/node/23127

Montes, I., Iriondo, M., Manzano, C., Arrizabalaga, H., Jiménez, E., Pardo, M. Á., Goñi, N., et al. 2012. Worldwide genetic structure of albacore *Thunnus alalunga* revealed by microsatellite DNA markers. Marine Ecology Progress Series, 471: 183–191.

Moore, B. R., Bell, J. D., Evans, K., Farley, J., Grewe, P. M., Hampton, J., Marie, A. D., et al. 2020. Defining the stock structures of key commercial tunas in the Pacific Ocean I: Current knowledge and main uncertainties. Fisheries Research, 230: 105525.

Takagi, M., Okamuraet, T., Chow, S., and Taniguchi, N. 2001. Preliminary study of albacore (*Thunnus alalunga*) stock differentiation inferred from microsatellite DNA analysis. Fishery Bulletin, 99: 697–701.

Teears, T., Castillo-Jordán, C. Davies, N., Day, J., Hampton, J., Magnusson, A., Peatman, T., Pilling, G., Xu, H., Vidal, T., Williams, P. and Hamer, P. 2024. Stock assessment of South Pacific albacore: 2024. SC20/SA-WP-02-Rev 3. Twentieth Regular Session of the Scientific Committee of the Western and Central Pacific Fisheries Commission, Manila, Philippines, 14-21 August 2024.

Tremblay-Boyer, L., Bravington M, Davies C (2024). Updated design models informing the sampling strategy for a close-kin mark-recapture application to South Pacific albacore. WCPFC-SC-20-2024/SA-IP-24. Twentieth Regular Session of the Scientific Committee of the Western and Central Pacific Fisheries Commission. Manila, Philippines, 14-21 August 2024. https://meetings.wcpfc.int/node/23132

Weir, B. S., & Cockerham, C. C. (1984). Estimating F-Statistics for the Analysis of Population Structure. *Evolution*, *38*(6), 1358–1370. https://doi.org/10.2307/2408641

Williams, A. J., Farley, J. H., Hoyle, S. D., Davies, C. R., and Nicol, S. J. 2012. Spatial and sex-specific variation in growth of albacore tuna (*Thunnus alalunga*) across the South Pacific Ocean. PLoS ONE, 7(6): e39318.

Appendix 1

Table A1. Detailed collection information (corresponding to Table 1 in methods section) for each Port sampling location by port, sampling year, pooled area label for analysis (Area Code), number of fish sampled (N), average length, and range of lengths for albacore tuna used in the current study.

Port Sampling Location		Area Code	N	avg. length	Length range
Eaglehawk Neck, Australia	2017	TAS	17	45	43 - 47
Eaglehawk Neck, Australia	2018	TAS	40	45	43 - 47
Vancouver, Canada[1]*	2023	USF	100	65.7	53 - 82
Juvenile troll fishery 1200nm due east of New Zealand					
Mooloolaba, Australia	2018	COR	46	89.6	81 - 96
Noumea, New Caledonia	2013	NC	27	95.5	78 - 104
Noumea, New Caledonia	2014	NC	37	94.1	84 - 106
Noumea, New Caledonia	2016	NC	40	96.5	82 - 105
Noumea (June), New Caledonia	2023	NC	48	95.2	88 - 102
Noumea (Nov), New Caledonia	2023	NC	60	96.6	89 - 105
Westport, New Zealand	2008	NZ	46	50.1	47 - 73
Westport, New Zealand	2010	NZ	45	70.6	45 - 98
Westport, New Zealand	2023	NZ	89	52.7	47 - 76
wSuva, Fiji	2024	FIJ	93	96	86 - 103
eSuva, Fiji	2024	FIJ	51	91.7	84 - 99
Nuku'alofa, Tonga	2024	TON	93	93.7	85 - 101
Apia, Samoa Cook Islands EEZ between Tokelau and Tautua	2024	TOK	92	93.4	85. - 107
Papeete (June), French Polynesia	2022	FP	31	N/A	N/A
Papeete (Nov), French Polynesia	2022	FP	60	93.8	84 - 105
Total			1867		

[1*]- the USF is US High Seas Fishery fishing approximately 1200 nautical miles east of Christchurch, New Zealand.

Appendix 2

Detailed DNA Extraction and Quality Control

In the laboratory, muscle tissue samples were placed into 96-well deep-well plates, and tissue lysis was carried out directly on the biopsy tips using Proteinase K and Buffer ATL, as provided in the Qiagen QIAamp 96 DNA QIAcube HT Kit (Cat. No. 51331; Qiagen, Hilden, Germany), following a modified version of the kit protocol. The samples were incubated overnight at 56°C to ensure thorough breakdown of the tissue and efficient release of nucleic acids.

Following tissue digestion, the lysates were centrifuged in their original 96-well plates to pellet any residual tissue debris. This step ensured that debris would not be transferred, thereby reducing the risk of clogging pipette tips or silica membranes during subsequent purification. The cleared lysates were then transferred to a new 96-well plate using the Integra ASSIST PLUS pipetting robot (INTEGRA Biosciences AG, Zizers, Switzerland), which provided consistent sample handling and reduced variability in the pipetting process.

DNA extraction was performed on the Hamilton Microlab STAR automated liquid handling platform (Hamilton Bonaduz AG, Bonaduz, Switzerland), equipped with dual on-deck vacuum stations. A custom script was developed specifically to enable ultra-high-throughput processing of the modified Qiagen QIAamp 96 DNA QIAcube HT Kit protocol (Cat. No. 51331; Qiagen, Hilden, Germany), allowing extraction of DNA from up to 960 samples per run. Each 96-well plate contained 92 experimental samples with muscle tissue from albacore tuna (ALB), 2 control samples from standard reference tissue (southern bluefin tuna (*Thunnus maccoyii*)), and 2 blank wells serving as negative controls.

Quality control (QC) testing was performed on 25% of the samples from each plate to evaluate DNA extraction efficiency—including DNA concentration, purity, and integrity—and to assess plate-level consistency. QC wells were selected from three representative columns to capture a spatial distribution across each plate that reflects the overall extraction performance. QC sample preparation was automated using the epMotion® 5075 automated liquid handling system (Eppendorf AG, Hamburg, Germany) to ensure reproducibility and reduce manual errors.

For each QC assay, 4µL of DNA was used from a total elution volume of 125µL. DNA concentration and purity was measured using the Multiskan SkyHigh spectrophotometer (Life Technologies Holdings Pte Ltd, Singapore), following a 1:10 dilution of each sample. DNA integrity was evaluated using the Invitrogen E-Gel™ Power Snap Plus Electrophoresis System (Life Technologies Holdings Pte Ltd, Singapore) with precast Invitrogen E-Gel™ 96 Agarose Gels 1% with SYBR™ Safe (Cat No. G720801; Thermo Fisher Scientific, Kiryat Shmona, Israel).

Although QC reliably measures extraction efficiency, it also reflects inherent biological variability at the plate level. This subset-based approach assumes that the average tissue quality within each extraction plate is representative, allowing meaningful inference of overall DNA quality across samples on that plate.

DNA Sequencing

Genomic DNA was submitted to Diversity Arrays Technologies (DArT) in Canberra where reduced representation library construction, sequencing and genotyping was completed using DArT PL's patented protocol, DArTseq™. Some procedures were proprietary, but reasonably detailed descriptions are available (Sansaloni et al. 2011; Kilian et al. 2012; Cruz et al. 2013; Ren et al. 2015). Adaptors that include variable barcode sequences and Illumina flowcell attachment sequences were ligated to fragments. PCR amplified only mixed fragments in a sequence of initial denaturation at 94°C for one minute, followed by 30 cycles of 94°C for 20 seconds, 58°C for 30 seconds, and 72°C for 45 seconds. A final extension step took 7 minutes

at 72°C. Libraries were bulked and applied to c-Bot bridge PCR, then single-end sequenced on an Illumina Novaseq6000 platform for 83 cycles.

Raw reads obtained following sequencing were processed using DArTech PL's proprietary analytical pipelines according to Kilian et al. (2012). The pipelines filter away poor-quality sequences, demultiplex reads, groom out singletons and other low-quality tags, and eventually apply DArTsoft14 variant calling algorithms. Single Nucleotide Polymorphism (SNP) markers were further filtered for paralogs, low read depth and suspect call quality. Based on this dataset, we flagged a variety of samples that were either not relevant to the current analysis or did not sequence normally. The remaining raw sequencing reads were then resubmitted to the DArTsoft14 pipeline to call genotypes that are study specific.