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Progress Towards a Close-Kin-Mark-Recapture Application to South Pacific Albacore (Project 100c)

WCPFC-SC20-2024/SA-WP-09

1 August 2024

SPC-OFP[1] and CSIRO[2]

[1] Oceanic Fisheries Programme (OFP), Pacific Community (SPC), Noumea, New Caledonia
[2] The Commonwealth Scientific and Industrial Research Organisation, Australia

Executive Summary

SC17 established Project 100c to prepare western and central Pacific tuna fisheries for the application of close-kin mark-recapture (CKMR) methods to resolve key stock assessment uncertainties.

The project is co-funded between WCPFC and the European Union with additional support from the Oceanic Fisheries Programme of the Pacific Community (SPC-OFP) and CSIRO, Australia. The financial contribution of the European Union is sourced through their European Maritime, Fisheries and Aquaculture Fund. EMFAF funds became available in November 2022 (~10 months later than originally planned at SC17). To accommodate this delay, project milestones have been extended by 12 months (as noted in SPC-OFP 2022 [SC18/SA-IP-10]).

A working paper to SC19 (SPC-OFP and CSIRO 2023 [SC19/SA-WP-03]) listed the specific activities of Project 100c and summarised work completed up until SC19. This working paper updates SC20 on progress under each activity over the past 12 months. Specific activities are listed below, with brief updates under each in italics. Detailed updates for each activity are found in the main text.

1. Complete the foundational research needed for the application of CKMR methods to WCPFC stocks to reduce the uncertainty in stock assessments. This will include:
    i. epigenetic ageing for south Pacific albacore and Pacific bigeye using existing validated otolith age.

    A calibrated epigenetic age model was developed for bigeye and yellowfin tuna in 2023 that covered ages ranging from 0.2 to 11 years and showed a high correlation between epigenetic methylation in muscle tissue and validated otolith age. The information is now being translated to a high-throughput platform in collaboration with Diversity Arrays Technology (DArT), Australia. An updated model will follow, incorporating additional otolith and muscle samples from large bigeye, if available. Epigenetic age calibration of south Pacific albacore tuna will be undertaken once the technology is transferred to DArT.

    ii. evaluation of radiocarbon otolith age validation of southwest Pacific swordfish and epigenetic age calibration.

    Radiocarbon age validation has commenced and ^{14}C results from 13 swordfish otoliths are now in hand. Additional samples are available from the CSIRO archives; however, the search for samples from larger, potentially older specimens continues..Epigenetic age calibration can commence once radiocarbon age validation has been fully developed, given that sufficient high quality muscle tissue samples from "known age" fish are available.

    iii. genome resequencing of south Pacific albacore and Pacific bigeye for enhanced detection of kin-pairs.

    Currently underway for a total of 36 individuals per species for albacore, bigeye, yellowfin and skipjack tuna. Sampling covers six locations across the Pacific region.

2. Complete CKMR feasibility and design study for South Pacific albacore.

    The design study has been updated (see Tremblay-Boyer et al. 2024 and Appendx 1 for full details) and the target sample size revised to 36,000-84,000 tissue samples across three years. Sampling feasibility studies and rollout to date indicate that achieving this target remains possible.

3. Complete CKMR scoping studies for Pacific bigeye and southwest Pacific swordfish.

   The Pacific bigeye scoping study is now complete. Results indicate that between 60,000 and 100,000 tissue samples will be needed over a three-to-four-year period to achieve acceptable levels of precision (~15% CV) in estimates of abundance. A formal design study is recommended to provide more precise estimates. The southwest Pacific swordfish scoping study has commenced and is expected to be completed before the end of 2024.

4. Develop and trial Standard Operating Procedures for the cost effective and reliable collection of tissue samples necessary for CKMR applications to WCPFC stocks.

   A core standard operating procedure (SOP) has been developed that can be adapted to the diverse sampling scenarios encountered at key ports. The core SOP has now been introduced to port samplers in Fiji, FSM, Samoa, RMI, Solomon Islands and Tonga via a new SPC-OFP training course. The first DNA quality control results are expected in August 2024 and will inform any refinements to the SOP that are required.

5. Use trial samples to investigate and validate connectivity hypotheses via non-close-kin methods for south Pacific albacore in preparation for the 2024 stock assessment.

   An analysis of genetic and otolith shape data (see Macdonald et al. 2024 and Appendix 2 for full details) provides strong evidence of population differentiation between New Caledonia and French Polynesia. These results align with modelled movement rate estimates used in the 2024 assessment, and together with other lines of evidence, lend support to the 2-region spatial structure adopted for the assessment. Follow-up work and further sampling is recommended to help clarify the precise location of any longitudinal division in the south Pacific albacore stock.

6. Develop capacity within WCPFC to implement and evaluate CKMR applications to WCPFC stocks.

   Six training events have been held in 2024 focused on SOP for port-based tissue sampling. These have upskilled 59 port samplers, fisheries observers, government and industry staff from 13 countries. In total, just over 11,000 tissue samples from south Pacific albacore have been collected since February 2023.

7. Provide advice to the Scientific Committee on what further research and data improvements are needed to enable best use of CKMR methods.

   This activity is scheduled for project completion.

Regarding detailed progress on activities 2 and 5, we refer readers to two companion papers designed to be read in conjunction with the current paper. The first, Tremblay-Boyer et al. (2024) [SC20/SA-IP-24], details progress on the CKMR design study for south Pacific albacore. The second, Macdonald et al. (2024) [SC20/SA-IP-04], presents findings from phase one of an investigation into population structure and regional connectivity of south Pacific albacore in the western and central Pacific Ocean (WCPO) and eastern Pacific Ocean (EPO). Copies of these two papers are also available in the Appendices to the current paper.

SC20 is invited to:

- note the progress on each activity to date;
- recall that all proposed project milestones are delayed by 12 months (from that endorsed at SC17) due to the EMFAF grant not commencing until November 2022;

- consider the scheduling of and resourcing for the inclusion of CKMR data in future stock assessments for south Pacific albacore, noting the demonstrated capacity of sampling teams now established throughout the region to achieve the updated target of 36,000-84,000 tissue samples over a three-year period (SC20/SA-IP-24); and
- acknowledge the European Union for their continued support of this work.

Specific to activity 2, regarding study design, SC20 is invited to:

- consider the updated sampling numbers summarized here and reported in Appendix 1 for the statistical sampling design for south Pacific albacore under Project 100c;
- consider supporting the genotyping of samples collected to date to narrow the range of population scenarios considered from the sampling design;
- consider supporting the investigation into the optimal allocation of ageing methods across ages to minimise ageing costs while improving overall ageing precision.

Specific to activity 5, testing connectivity hypotheses, SC20 is invited to:

- Note the Phase 1 results summarised in this paper and presented in Appendix 2.
- Support the 2024 PAW recommendation for follow-up studies of south Pacific albacore population structure, including completion of the otolith microchemistry component of Phase 1 and refinement of a Phase 2 design, that:
    i) incorporates finer-scale, structured sampling across the WCPO and further east in the EPO;
        – We note that PAW 2024 highlighted the opportunity for EPO sampling by members that operate vessels in that jurisdiction.
        – We invite SC20 to encourage those members to participate in the necessary sample collection, as well as request SPC-OFP to liaise with the IATTC to enable opportunities for collaborative sample collection.
    ii) combines empirical and modelled data from a variety of sources where available; and
    iii) explores intrinsic and environmental mechanisms that might give rise to the observed population structure.
- Recognise the value of multiple lines of evidence, as presented here, to:
    i) help inform decisions on spatial structure in tuna stock assessments (sensu Hamer al. 2023); and
    ii) help inform CKMR sampling designs and analytical pipelines for WCPFC Project 100c.

Background

A significant challenge for several WCPFC stocks assessments is estimating the absolute spawning biomass with the necessary accuracy and precision to assist management decision making. Application of close-kin mark-recapture (CKMR) methods offers the most practical solution to resolve this challenge. Every animal is born with exactly one living mother and one living father, which it "marks" genetically. CKMR takes advantage of this and modern genotyping methods to identify pairs of close relatives (e.g. parent-offspring, half-brother-sister). The number of kin-pairs found, and the way they are distributed in space and time, can be embedded into a population dynamics model and used to

estimate absolute adult abundance, as well as other important demographic parameters such as mortality rates, fecundity and connectivity. Unlike conventional mark-recapture methods, CKMR uses tissue biopsies taken from individuals captured by the fishery. Other important information on fish age, fish sex and population structure can, potentially, also be obtained from the biopsies obtained and genotype information, providing significant efficiencies to fisheries monitoring programmes.

The successful application of CKMR is dependent on adequate understanding of a species' biology, fishery operation and consideration of sampling logistics. This includes the capacity to collect enough tissue samples, given the size of the stock, to identify sufficient numbers of kin pairs and the capacity to estimate the age of the individuals sampled. Validating our understanding of the species biology (e.g., reproductive biology, sexual dimorphism, spatial connectivity) and evaluating the logistical feasibility of sample collection, storage, transport and analysis are necessary first steps for implementing CKMR.

Project 100c was established by the WCPFC Scientific Committee (SC) at SC17 to address these questions. The specified activities of the project include:

1. Complete the foundational research needed for the application of CKMR methods to WCPFC stocks to reduce the uncertainty in stock assessments. This will include:
    i. epigenetic ageing for south Pacific albacore and Pacific bigeye using existing validated otolith age.
    ii. evaluation of radiocarbon otolith age validation of southwest Pacific swordfish and epigenetic age calibration.
    iii. genome resequencing of south Pacific albacore and Pacific bigeye for enhanced detection of kin-pairs.
2. Complete CKMR feasibility and design study for South Pacific albacore.
3. Complete CKMR scoping studies for Pacific bigeye and southwest Pacific swordfish.
4. Develop and trial Standard Operating Procedures for the cost effective and reliable collection of tissue samples necessary for CKMR applications to WCPFC stocks.
5. Use trial samples to investigate and validate connectivity hypotheses via non-close-kin methods for south Pacific albacore in preparation for the 2024 stock assessment.
6. Develop capacity within WCPFC to implement and evaluate CKMR applications to WCPFC stocks.
7. Provide advice to the SC on what further research and data improvements are needed to enable best use of CKMR methods.

Project Administration

This project is supported by the European Union through its European Maritime, Fisheries and Aquaculture Fund with a budget of approximately Euro 270,000. WCPFC has allocated a further USD40,000 to support implementation. SPC-OFP and CSIRO are providing additional in-kind and operational support (to December 2025). Project review and guidance is provided by the SC.

Progress to date on each activity

Activity 1 — Complete the foundational research needed for the application of CKMR methods to WCPFC stocks to reduce the uncertainty in stock assessments.

i—epigenetic ageing for south Pacific albacore and Pacific bigeye using existing validated otolith age.

A calibrated epigenetic age model was developed for bigeye and yellowfin tuna in 2023 through an ACIAR-CSIRO project. The model covered ages ranging from 0.2 to 11 years and showed a high correlation between epigenetic methylation in muscle tissue and validated otolith age. A high-throughput method to determine tissue methylation levels is being planned in collaboration with Diversity Arrays Technologies (DArT) in Canberra, Australia, using the same set of genetic markers. Once complete, tissue from the "known age" calibration set will be sequenced at DArT, and a new model will be developed. Additional otoliths and muscle tissue for bigeye tuna tissue may be obtained from the Pacific Marine Specimen Bank (PMSB) for inclusion in the new calibration process, particularly if tissue from larger/older fish are present. Similarly, epigenetic age calibration of south Pacific albacore tuna will be undertaken once the technology is transferred to DArT.

ii—evaluation of radiocarbon otolith age validation of swordfish and epigenetic age calibration.

The otolith radiocarbon age validation work has commenced for southwest Pacific swordfish. As of 25 July 2024, 13 swordfish otoliths drawn from the Pacific Marine Specimen Bank (PMSB) archive in Noumea have been cored to isolate material formed during early life and the resulting powders analysed for ^{14}C at the Research School of Earth Sciences at the Australian National University in Canberra. The sister otoliths are currently with Fish Ageing Services in Melbourne and are being prepared for annual age estimation. CSIRO has additional otoliths available for radiocarbon analysis, including 125 otoliths that have no associated age estimate as yet, and 194 sister otoliths of the 301 swordfish aged by Farley et al. (2022). These range in estimated birth years from 1979 to 2001; however, most are from 1992 to 1997. The search for otoliths continues, particularly those from larger, potentially older specimens for which the main data gap exists.

Preliminary epigenetic age calibration of swordfish can be undertaken if otolith age is validated using the radiocarbon method and there are sufficient high quality muscle tissue samples available from "known age" fish.

iii— genome resequencing of south Pacific albacore and Pacific bigeye for enhanced detection of kin-pairs.

Genome resequencing is currently underway for 6 individuals from four tuna species (albacore, bigeye, yellowfin and skipjack tuna) from each of six selected sampling regions (e.g. Coral Sea, Guam, Marshall Islands, French Polynesia, Hawaii, and California/Eastern Pacific). This information will be used to guide marker selection and determine loci to be used for CKMR.

Activity 2 — Complete CKMR feasibility and design study for south Pacific albacore.

Through updates to the CKMR design work for south Pacific albacore (described in detail in SC20-SA-IP-24, provided in Appendix 1), in particular, the incorporation of data from the 2021 and 2024 albacore stock assessments (Castillo-Jordán et al., 2021, Teears et al. 2024), a new sampling target has been estimated at 36,000-84,000 tissue samples over three years which would allow to achieve CVs of 15% or less on estimates of recent biomass of adults (4 years and older) in the WCPO (assuming minimum connectivity with the EPO, see SC20/SA-IP-04, provided in Appendix 2).

This wider range in sampling target than previously estimated (Bravington et al. 2021) is deemed to be more representative of the unknown population scaling for the stock. It is driven by recent changes in assessment methodology which reflect uncertainty about some key biological parameters, such as stock structure and natural mortality. Instead of using one stock assessment as a baseline to generate sampling targets (as in Bravington et al. 2021), the 2018, 2021 and 2024 south Pacific albacore assessments were considered to represent different population scenarios, and the updated sampling target reflects these possible states of nature.

In addition, early genotyping of samples collected to date might narrow the range of likely population scenarios to focus on when informing evolving sampling numbers. Increasing sampling program length by a year can also allow similar objectives to be met (in terms of the target precision for CKMR modelling) while reducing annual numbers.

With regards to feasibility, owing to the successful onboarding of new staff and contractors at key sampling ports across the WCPO, the training events conducted during 2024 and sample numbers collected to date (see Tables 2-4 under Activity 6 for full details) there is demonstrated capacity of the regional sampling teams to achieve the updated sampling target over a two-to-three-year period. Work is also underway through WCPFC Project 90 to develop a fork length to tail measurement conversion factor for south Pacific albacore (Macdonald et al. 2024). This will allow for accurate fork length (and hence age) estimates to be derived from tail cuts alone, supporting the south Pacific albacore CKMR sampling programme for fish landed into US and Canadian ports.

Activity 3 — Complete CKMR scoping studies for Pacific bigeye and southwest Pacific swordfish.

A CKMR scoping study for Pacific bigeye tuna has been completed. The scoping study used population parameters and abundance estimates from the diagnostic model in the most recent WCPFC bigeye tuna stock assessment (Day et al. 2023) to estimate the number of parent-offspring pairs (POPs) and half-sibling pairs (HSPs) that would be expected to be identified from alternative scenarios of abundance, annual sample sizes and sampling program lengths. The two alternative abundance scenarios examined were i) the estimate of numbers-at-age from the terminal year of the diagnostic model of the 2023 stock assessment, and ii) twice the numbers-at age from this model as an extreme estimate of the real population size. For simplicity, we assumed the ratio of adult to juvenile sampling was 50:50.

Results (Table 1 below) indicate that under the assumption that abundance estimates from the 2023 stock assessment are accurate, at least 60,000 samples of bigeye tuna (e.g. 15,000 in each of 4 years, or 20,000 in each of 3 years) would be required to detect at least 100 kin pairs (POPs and HSPs

combined), which is considered the minimum number required to achieve acceptable levels of precision (~15% CV) in estimates of abundance. Alternately, if the real abundance was much larger (i.e. twice the 2023 stock assessment estimate) then approximately 100,000 samples would be required to detect at least 100 kin pairs.

A proper design study, such as the study recently completed for south Pacific albacore (SC20/SA-IP-24, Appendix 1), that represents the biology more accurately and considers alternative abundance assumptions and sampling options, should be completed to provide more precise estimates of the sampling requirements to achieve the required precision in population estimates.

A CKMR scoping study for southwest Pacific swordfish has commenced but has not been completed due to delays in compiling the required information for the analysis. The CKMR scoping study for southwest Pacific swordfish is expected to be completed before the end of 2024 and an extension until this time is requested to allow completion of this work.

Table 1. Results from the CKMR scoping study for Pacific bigeye tuna.

Abundance scenario	Annual samples	Sampling years	POPs	HSPs	Total samples	Total Kin
Stock assessment	10000	3	5	22	30000	27
Stock assessment	10000	4	10	40	40000	50
Stock assessment	10000	5	16	63	50000	78
Stock assessment	15000	3	12	50	45000	62
Stock assessment	15000	4	22	90	60000	112
Stock assessment	15000	5	35	141	75000	176
Stock assessment	20000	3	20	89	60000	110
Stock assessment	20000	4	39	160	80000	198
Stock assessment	20000	5	63	250	100000	313
Stock assessment	25000	3	32	140	75000	172
Stock assessment	25000	4	60	249	100000	310
Stock assessment	25000	5	98	391	125000	489
2x Stock assessment	10000	3	3	11	30000	14
2x Stock assessment	10000	4	5	20	40000	25
2x Stock assessment	10000	5	8	31	50000	39
2x Stock assessment	15000	3	6	25	45000	31
2x Stock assessment	15000	4	11	45	60000	56
2x Stock assessment	15000	5	18	70	75000	88
2x Stock assessment	20000	3	10	45	60000	55
2x Stock assessment	20000	4	19	80	80000	99
2x Stock assessment	20000	5	31	125	100000	156
2x Stock assessment	25000	3	16	70	75000	86
2x Stock assessment	25000	4	30	125	100000	155
2x Stock assessment	25000	5	49	195	125000	244

Activity 4 — Develop and trial Standard Operating Procedures (SOP) for the cost effective and reliable collection of tissue samples necessary for CKMR applications to WCPFC stocks.

The SOP for genetic sampling of tuna and pelagic species for CKMR studies was drafted in 2022 and first trialled in New Zealand in February 2023. The SOP includes information on sample collection, preservation, storage and transport and has undergone further refinement for the Pacific port context in early 2024. It is appropriate to acknowledge the diversity of sampling conditions and logistical challenges present at the different ports, which has limited the creation of a single SOP document covering all ports and sampling situations. Instead, the most fundamental procedures have now been synthesised into a 'core protocol', common to all ports, which is then tuned to specific port scenarios. The SOP has been introduced to port samplers via a new SPC training course on port-based tissue sample collection which has now been presented in Fiji, FSM, Samoa, RMI, Solomon Islands and Tonga (Table 2 under Activity 6). Each training event includes modules to help adapt the core protocol to the port where the training is being held.

The first batches of tissue samples collected by in-country staff using the SOP have now been shipped to CSIRO in Hobart, Tasmania, and submitted for DNA sequencing and assessment for quality control (QC) purposes. The results of these QC analyses are expected in August 2024; the results will provide a basis to measure the success of all aspects of the SOP and training processes and to make further refinements if needed.

It is important to note that these samples were transported with the intention to expedite the QC work – the environmental conditions they experienced and the time spent in transit potentially not reflecting standard shipping processes in some Pacific ports. Optimising transport operations from each sampling port to maintain DNA quality is a priority for CKMR studies and this is an ongoing area of work for Project 100c. We highlight that a system for continuously monitoring the sample environment during transit is being explored in conjunction with an investigation into the shipping routes and packaging styles that will ensure the most efficient maintenance of the cold chain for frozen samples during transport.

Activity 5 — Use trial samples to investigate and validate connectivity hypotheses via non-close-kin methods for south Pacific albacore in preparation for the 2024 stock assessment.

A study was initiated in late 2022 to better define the population structure of south Pacific albacore across the WCPO and EPO. The study takes a multidisciplinary approach using population genetics analysis coupled with analyses of otolith morphology and otolith microchemistry. The specific aims are to i) help inform decisions on the spatial structure for the 2024 Pacific-wide south Pacific albacore assessment (Teears et al. 2024), along with other supporting analyses (Potts et al. 2024), and ii) help guide sampling strategies and analytical pipelines for Project 100c. The results of Phase 1 of the work, involving a broad-scale comparison between the western WCPO (New Caledonia) and the western EPO (French Polynesia), are presented in full in SC20/SA-IP-04 and provided in Appendix 2. In summary, analyses of otolith shape and genetic data from sexually mature individuals captured within the New Caledonian EEZ (n = 55) and the French Polynesian EEZ (n = 55) in November 2022 both support the presence of population structure in south Pacific albacore between the two sampling locations. Genetic data from an additional 38 individuals captured from New Caledonian waters in June 2022 indicate seasonal stability in the New Caledonian population genomic signature over at least a 5-month period. Therefore, seasonal variation cannot explain the genetic differentiation observed between fish collected in New Caledonia and French Polynesia. SPC-OFP have invited SC20

to support the recommendation from the 2024 pre-assessment workshop (PAW) (Hamer 2024) for follow-up studies of south Pacific albacore population structure, including a second phase of this project that proposes finer-scale sampling be undertaken across the WCPO and further east in the EPO during 2024 and 2025.

Activity 6—Develop capacity within WCPFC to implement and evaluate CKMR applications to WCPFC stocks.

SPC-OFP staff have led six training events in 2024 focused on SOP for port-based tissue sampling (as this is the main protocol employed in CKMR sampling for south Pacific albacore). In total, these events have upskilled 59 port samplers, fisheries observers, government and industry staff from 13 countries, each of whom are now qualified to independently collect tissue samples for CKMR studies. Note that 21 workshop attendees in Fiji received theory-only training, four of whom have continued with practical training and are included in the final list of trained staff. See Table 2 for a breakdown of training event attendance.

Table 2. Summary of training events for the port-based tissue sampling SOP as of 1 July 2024.

Training	Date	Trainees	Representatives per country
Fiji, Suva	Jan-24	21*	Fiji - 21
FSM, Kosrae	May-24	5	FSM-5
RMI, Majuro	Mar-24	8	RMI – 3 Nauru – 1 FSM – 2 Kiribati - 2
Samoa, Apia	Mar-24	11	French Polynesia – 2 Samoa – 2 Cooks – 2 Vanuatu – 2 Tuvalu - 2
Solomon Islands, Noro	Apr-24	17	Soloman Islands - 17
Tonga, Nuku'alofa	Feb-24	10	Tonga - 10

*21 staff attended the workshop in Fiji and received all theoretical training modules. Of these, four are now fully trained and 17 only need to complete practical demonstrations.

In addition, two individuals in New Caledonia and three individuals in New Zealand have received full training outside the formal workshop environment and are likewise qualified to lead port-based tissue sampling events. See Table 3 for a by-country breakdown of fully trained staff.

Table 3. Staff per country fully trained in the port-based tissue sampling SOP as of 1 July 2024.

Country	Staff trained
Cook Islands	2
Fiji	4
French Polynesia	2
FSM	7
Kiribati	2
Nauru	1

New Caledonia	2
New Zealand	3
RMI	3
Samoa	2
Solomon Islands	17
Tonga	10
Tuvalu	2
Vanuatu	2
Total	**59**

Between sampling events conducted by the abovementioned staff and those conducted by SPC-OFP staff, just over 11,000 tissue samples from south Pacific albacore have been collected since February 2023 (Table 4). Over 8,600 of these were collected in the first half of 2024, reflecting the recent expansion of sampling capacity across the region. Approximately two thirds of the samples collected to date have come from juveniles aged between 1 and 3 years old captured in the New Zealand troll fishery, with samples in other ports being sourced from longline vessels that generally target larger individuals (Table 4). Metadata associated with each sample (i.e. fish length, sample label number, sampling location on the fish, other comments) are uploaded to the SPC's Tufman2 and BioDaSys databases via the 'OnShore' data entry application used by the sampling teams in port. We note that the past 12 months has seen substantial refinements to OnShore's data entry layout in response to feedback from the CKMR sampling teams, and these have done much to improve sampling efficiency at port.

Table 4. Numbers of tissue samples collected for the CKMR study on south Pacific albacore by port, as queried from the Tufman2 database on 16 July 2024.

Port	2023	2024	Total
Fiji, Suva	0	1087	1087
New Caledonia, Noumea	46	188	234
New Zealand, Greymouth	1073	4266	5339
New Zealand, Westport	1327	492	1819
Samoa, Apia	0	108	108
Solomon Islands, Noro	0	1171	1171
Tonga, Nuku'alofa	0	1297	1297
Total	**2446**	**8609**	**11055**

Activity 7—Provide advice to the Scientific Committee on what further research and data improvements are needed to enable best use of CKMR methods.

This activity is scheduled for project completion.

Recommendations

SC20 is invited to:

- note the progress on each activity to date;
- recall that all proposed project milestones are delayed by 12 months (from that endorsed at SC17) due to the EMFAF grant not commencing until November 2022;
- consider the scheduling of and resourcing for the inclusion of CKMR data in future stock assessments for south Pacific albacore, noting the demonstrated capacity of sampling teams now established throughout the region to achieve the updated target of 36,000-84,000 tissue samples over a three-year period (SC20/SA-IP-24); and
- acknowledge the European Union for their continued support of this work.

Specific to activity 2, regarding study design, SC20 is invited to:

- consider the updated sampling numbers summarized here and reported in Appendix 1 for the statistical sampling design for south Pacific albacore under Project 100c;
- consider supporting the genotyping of samples collected to date to narrow the range of population scenarios considered from the sampling design;
- consider supporting the investigation into the optimal allocation of ageing methods across ages to minimise ageing costs while improving overall ageing precision.

Specific to activity 5, testing connectivity hypotheses, SC20 is invited to:

- Note the Phase 1 results summarised in this paper and presented in Appendix 2.
- Support the 2024 PAW recommendation for follow-up studies of south Pacific albacore population structure, including completion of the otolith microchemistry component of Phase 1 and refinement of a Phase 2 design, that:
    iv) incorporates finer-scale, structured sampling across the WCPO and further east in the EPO;
        – We note that PAW 2024 highlighted the opportunity for EPO sampling by members that operate vessels in that jurisdiction.
        – We invite SC20 to encourage those members to participate in the necessary sample collection, as well as request SPC-OFP to liaise with the IATTC to enable opportunities for collaborative sample collection.
    v) combines empirical and modelled data from a variety of sources where available; and
    vi) explores intrinsic and environmental mechanisms that might give rise to the observed population structure.
- Recognise the value of multiple lines of evidence, as presented here, to:
    i) help inform decisions on spatial structure in tuna stock assessments (sensu Hamer al. 2023); and
    ii) help inform CKMR sampling designs and analytical pipelines for WCPFC Project 100c.

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Appendix 1: SC20/SA-IP-24

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Updated design models informing the sampling strategy for a close-kin mark-recapture application to South Pacific albacore

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29 July 2024

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Important disclaimer

1 Executive summary

Project 100c was established by SC 17 to assess the feasibility of applying close-kin mark-recapture in the Western and Central Pacific Commission, starting with south Pacific albacore as a trial population. Initial design studies based on the 2018 stock assessment for this population provided first estimates of the number of individuals that should be sampled in order to achieve an estimate of adult biomass (4 years and older) that would be useful for management (CV ¡ 15%). These numbers are updated here to account for the two most recent assessments for this stock and correct an issue with the initial data inputs.

The sampling design model used the same framework as that developed by Bravington et al. (2021) (SC17-SA-IP-14) but explored alternative population scenarios to account for the changes in assessment methodology and assumed stock structure since 2018. The impact of sampling program length and the proportion of juvenile to adult samples were also examined. Depending on the population scenario used, the sampling numbers required to achieve sufficient precision on adult biomass estimates from a CKMR model vary from 36,000 to 84,000 tissue samples collected over three years. This wide range reflects the variability in the numbers-at-age predicted by recent stock assessments for south Pacific albacore and uncertainty about stock structure and key life-history parameters. Genotyping the samples collected to date might provide further insights as to the most likely population scenario and reduce the range of samples needed according to the statistical design. Also, the numbers of samples to be collected annually could be reduced if the sampling program is extended to a fourth year.

Recommendations

SC 20 is invited to:

- consider these updated sampling numbers for the statistical sampling design for south Pacific albacore under Project 100c;
- consider supporting the genotyping of samples collected to date to narrow the range of population scenarios considered from the sampling design;
- consider supporting the investigation into the optimal allocation of ageing methods across ages to minimise ageing costs while improving overall ageing precision.

2 Introduction

Close-kin mark-recapture (CKMR) is a fisheries-independent approach that can provide an estimate of absolute abundance, total mortality, and other key population metrics (Bravington et al., 2016). It can also yield insights about spatial connectivity within a population. In short, CKMR treats observations of kin pairs (genetically related individuals, such as parents and offsprings) as tag recaptures (an offspring 'tags' its parents), and the likelihood of observing a set number of kin pairs given population size, age and sex structure is calculated. This likelihood can then be used on its own in a population dynamics model to estimate the parameter vector that maximizes the likelihood given observations, or used in an integrated stock assessment framework in combination with other data sources. To observe kin pairs, tissue samples of a pre-specified number of individuals are collected from the population of interest. The samples are then genotyped and examined for evidence of genetic relatedness.

When using CKMR, precise estimates of key parameters can only be produced if enough samples are collected from the population across suitable age groups. As such, before implementing CKMR, it is important to undertake a design phase to assess the number of samples needed and their age distribution given a target precision in parameter estimates (typically a CV $<$ 15%, but this can be modified depending on preference). In general, the larger the number of individuals collected, the better precision on the resulting model-derived estimates of population metrics. Apart from information on the biology and life-history of the species, the design phase requires that an assumption be made about the likely true population size. If the true population size is lower than the assumed size, then CKMR will provide abundance estimates with a precision that is greater than the target precision. If the true population size is larger than assumed, then the CKMR estimate of abundance will be less precise, but it will reveal that the stock is larger than what was assumed during the design study.

Project 100c was established by SC 17 to assess the feasibility of applying CKMR in the Western and Central Pacific Commission (WCPFC), starting with south Pacific albacore (*Thunnus alalunga*) as a trial population. Initial design studies (Bravington et al. 2020, followed by an update in Bravington et al. 2021) provided early estimates of the number of individuals that should be sampled in order to achieve an estimate of adult biomass (4 years and older) that would be useful for management (CV $<$ 15%)[1]. However, these studies used the 2018 stock assessment (Tremblay-Boyer et al., 2018) as a baseline for population dynamics, which means that sampling numbers were optimized for a population assumed to match the most recent period in the stock assessment (i.e., years 2014 to 2016). There have been two updates to the south Pacific albacore stock assessments since which have made changes to stock structure (most notably by extending the stock to include the Eastern Pacific ocean) and assumptions about life-history, both of which impact the best estimate of population dynamics in the recent period. As such, this Information Paper to SC 20 presents an updated sampling design for south Pacific albacore based on the most recent stock assessment for this population. Of note, the basic modelling framework developped under Bravington et al. (2021) was used here, including assumptions about the spatial structure of juveniles vs. adults. Issues regarding the treatment of some of the data inputs have also been corrected.

3 Methods

3.1 Overview

The statistical sampling design requires a population model that approximates 'recent' population dynamics for the stock, as that is what is effectively being targeted by the sampling program. Ideally, the most recent stock assessment for the population of interest would be used as a baseline. However, there were important changes in the methods and assumptions about stock structure in each of the three most recent stock assessments for south Pacific albacore, and these have impacted the scaling of predicted population numbers over similar time periods.

The 2018 stock assessment (Tremblay-Boyer et al. 2018; final prediction year 2016) was used for the most recent sampling design model (Bravington et al. 2021), and has guided sampling targets to date for the pilot sampling program under Project 100c. This assessment assumed the stock was fully contained within the southern WCPFC convention area. That assumption was

[1] about 25,000 individuals over three years, evenly spread between adult and juveniles

modified in the 2021 stock assessment (Castillo Jordán et al. 2021; final prediction year 2019) with the extension of the stock to the eastern Pacific ocean. The most recent stock assessment (Teears et al. 2024; final prediction year 2022) also assumed a stock spanning the southern Pacific ocean, but modifications to assumptions about natural mortality resulted in much higher numbers-at-age across similar time periods compared to previous assessments. To account for this, the current close-kin sampling design was updated for each stock assessment separately with the assumption they represented different population 'scenarios', or states of nature.

All data inputs were derived directly from the assessments' diagnostic case input and output files made available by SPC in the public domain[2], including total catches by fleet, numbers-at-age, and maturity and weight-at-age ogives. As the 2024 stock assessment had not been published at the time of the analyses presented here, a draft version of the diagnostic case was provided by SPC (J. Hampton, *pers. comm*, July 2024).

There are several considerations in the close-kin design approach to allow for uncertainty with regards to the spatial structuring of the south Pacific albacore stock (see Bravington et al. 2021 for a discussion). The inclusion of a scaling parameter in the design population model to represent the proportion of the juvenile population accessible by the sampling program was retained here. In addition, while main sampling numbers are presented for a population assumed to span the WCPFC convention area (including the overlap area), alternative scenarios where the stock spans the southern Pacific ocean are also included for the 2021 and 2024 stock assessments.

3.2 Catch-at-age

Catch-at-age distributions were built for each fleet from the length observations. The growth curve from the assessment was used to simulate the probability of observing an age from a given length (based on the standard deviation of lengths-at-age). Length records for each fleet were aggregated by 1cm bin class, based on the last five years for which length records were available for the fleet. These were assumed to be representative of a recent catch-at-age distribution for this fleet. Records by length bin were spread across possible age bins, rounded to the nearest integer, and summed across age bins by fleet. The proportion-at-age was obtained as the ratio of predicted observations by age bins to the total length observations for the fleet. The catch-at-age distribution for the fleet was then the product of this proportion-at-age vector and the average catch for the fleet over the last three years.

Only fleets active in the last three years of the assessment were retained. For active fleets missing sufficient recent length samples to approximate a catch-at-age distribution, the proportional catch-at-age distribution of a similar fleet was used. 'Similar' fleets were chosen to prioritize those using the same gear in the same stock assessment region.

3.3 Numbers-at-age and life-history

To approximate recent population dynamics, 'target' numbers-at-age for the simplified design model were specified as the annual average for the most recent period spanned by each of the three stock assessments. For the 2018 and 2024 assessments, a 'recent' period of three years from the final assessment year was used (i.e., 2014 to 2016 for the 2018 stock assessment

[2] https://oceanfish.spc.int/en/publications/cat_view/116-ofp-publications-a-documents/131-stock-assessment-and-modelling/181-stock-assessments/229-input-and-result-files

and 2020 to 2022 for the 2024 stock assessment). For the 2021 stock assessment, a 'recent' period of two years was used (i.e. 2018 and 2019) to avoid using population predictions adjacent to 2016, given concerns raised at previous WCPFC SC18 and SC19 with regards to the recruitment predictions made for that year (e.g., see Scott et al. 2023). For seasonal assessment models (2018 and 2021), annual numbers-at-age were first averaged over seasons. The resulting numbers-at-age vectors are shown in Figure 1 for both the recent period and an overlap period of 2014 to 2016 to showcase the difference in population scaling.

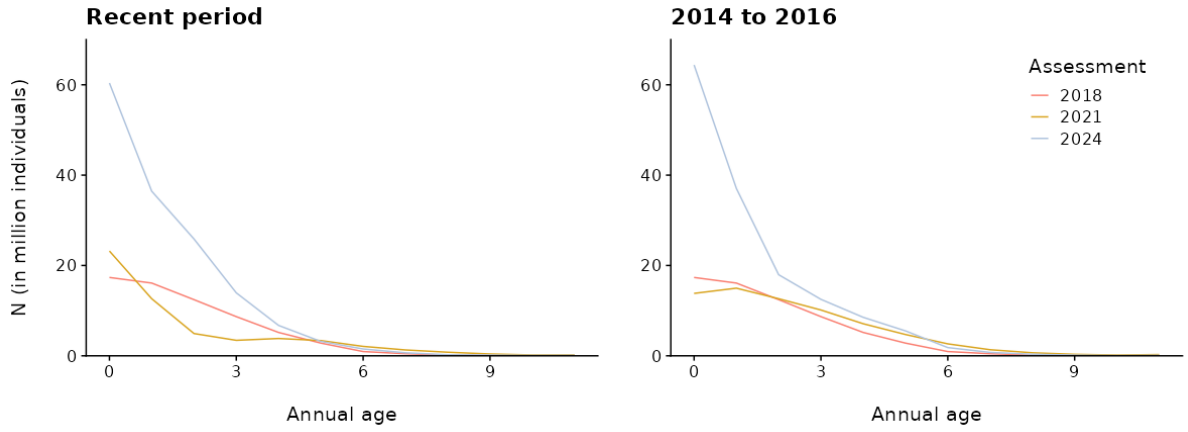

Figure 1: Numbers-at-age for the 'recent' (left panel) and an overlap (right panel) time period of each of the stock assessment scenarios (in colours) used in the statistical sampling design.

For consistency, maturity-at-age and weight-at-age ogives were set to the values used by Bravington et al. (2021) based on the 2018 stock assessment.

3.4 Population model

A simple age-structured population model was developed using the same framework as in Bravington et al. (2021). For each stock assessment scenario, the model was initialized using the target numbers-at-age vector described above (i.e., that from the corresponding stock assessment, converted to annual age bins where needed), and annual numbers-at-age were projected over a period of ten years. To avoid the need for a plus-group (which complicates the calculation of kin probabilities), the maximum age is set to 15 and the stock assessments' plus-group (at age 12) is distributed in the remaining age bins assuming a log-linear relationship between numbers and age. The population was then sampled by the sampling program over the last three years of that period, with samples allocated to juveniles (0 to 3 years) and adults (4 years and older). The model is sex-structured, assuming a sex ratio of 1:1. As dynamics are the same for males and females, the description below shows dynamics by time and age only. Recruitment to the first age class is assumed constant throughout. Model parameters are described in Table 1.

The initial population-at-age is set to that of the stock assessment. For subsequent years:

$$N_{t \in 2:Y,\, a=1} = R_0 \cdot \sigma_{R_t}$$
$$N_{t \in 2:Y,\, a+1} = N_{t,a} \cdot e^{-(F_a+M)}$$

where a is the age bin, R_0 (in numbers of fish) is calculated from B_0 using weight-at-age and

Table 1: Description of model parameters.

Symbol	Description
M	Natural mortality
α	Negative slope of the log-linear numbers-at-age relationship (i.e., Z, total mortality)
w_s	Scaling parameter by sex for the fecundity vs. weight relationship
B_0	Mean recruitment (by weight) in the first year
σ_R	Annual recruitment deviations
HS_P	Proportion of juvenile population targeted by sampling program

the theoretical numbers-at-age distribution given α, and F_a is solved for iteratively at each model step using assumed catch-at-ages and the Baranov equation.

Finally, to estimate the variance in the distribution of recruitment deviations (used when computing the model's covariance matrix, see below), a linear model of numbers as a function of age was first fitted assuming a quasi-poisson error distribution (with log-link) to the target numbers-at-age distribution. The variance of the model residuals was extracted and assumed equal to the variance of the log recruitment deviation.

3.5 Estimation of CVs for population metrics

The estimation of CVs for quantities computed within a CKMR population modelling framework consisted of three main steps:

Step 1: Population dynamics for south Pacific albacore were estimated as described above. For close-kin computations, three resulting quantities are especially important: numbers-at-age ($N_{t,a,s}$, abundance at time t, age a and sex s), survival-at-age (from natural and fishing mortality), and Total Reproductive Output (TRO) for the population at time t:

$$TRO_{t,s} = \sum_{a=0}^{A} N_{t,a,s}\varphi_{a,s}$$

where $\varphi_{a,s}$ is fecundity-at-age a and sex s, defined by a power relationship between weight-at-age W_a and the reproductive output by sex coefficient w_s, scaled by maturity-at-age m_a:

$$\varphi_{a,s} = m_a \cdot W_a^{w_s}$$

Step 2: Given population dynamics estimated in the first step, compute the probability of observing a Parent-offspring Pair (POP) or a Half-sibling Pair (HSP) for each pairwise combination of individual samples, as a function of the year they were sampled, their age at sampling and, for POPs only, the sex of the parent, for a given number of samples. The following assumptions are made:

- Age is known exactly for both adults and juveniles;
- Population dynamics (selectivity, maturity, fecundity, length) vary across age only, with no variability in individual length-at-age;

- POPs and HSPs are computed but grandparent-grand offspring pairs (GGPs) are not explicitly modelled.

The probability that an individual of age a_i captured in year t_i is the parent of an individual born in year c_j is defined as:

$$\mathbb{P}\left(POP \,|\, \{i,j\}\right) = \mathbb{I}(c_j < t_i < c_j + a_i) \times \frac{\varphi_{a_i-(t_i-c_j),s_i}}{TRO_{c_j,s_i}}$$

which is the ratio of the fecundity of a possible parent of that age in the year that the juvenile was born and the TRO of the population in that year. This probability is computed if the possible offspring was born before the possible parent was captured (given lethal sampling), and if the possible offspring was born after the possible parent was mature. Otherwise, it is set to zero.

The probability that an individual i born in year c_i is the half-sibling of an individual i' born in year $c_{i'}$ is the probability that an unobserved individual of unknown age would have been the parent of offspring born in two different cohorts separated by the difference in birth years between the cohorts ($\delta_i = c_{i'} - c_i$):

$$\mathbb{P}\left(HSP \,|\, \{i,i',s\}\right) = \frac{1}{HS_P} \cdot \sum_a \left(\frac{N_{c_i,a,s}\varphi_{a,s}}{TRO_{c_i,s}} \times \left[\frac{N_{c_{i'},a+\delta_i,s}}{N_{c_i,a,s}} \right] \times \frac{\varphi_{a+\delta_i,s}}{TRO_{c_{i'},s}} \right)$$

The first term computes the probability that the unobserved parent was the parent of the older juvenile, the second term computes the probability that the unobserved parent survived from the birth year of the older juvenile to the birth year of the younger juvenile (given natural and fishing mortality), and the third term computes the probability that the unobserved parent was in fact the parent of the younger juvenile given its reproductive output in that birth year. This is integrated over all possible parent ages, as we do not know the actual age of the unobserved parent, and repeated for both potential parent sexes (shared parent sex will be known from mitochondrial DNA), and scaled by HS_P, the proportion of the juvenile population accessed by the sampling program.

For HSPs, same cohort comparisons (i.e. individuals born in the same year) are not performed to avoid possible bias from 'good years' that could result in an excess of HSPs born in the same year.

Finally, a false-negative probability of detecting a HSP of 15% is assumed.

Step 3: Once the probabilities of observing a POP and observing a HSP for each combination of sampled individuals has been predicted by the model, the expected covariance matrix of the input parameter vector is computed and used to approximate the variance for quantities of interest using the delta method. This process is equivalent to simulating a large number of input datasets, fitting the CKMR model to each one, and calculating the variance in the resulting estimated quantities (see Bravington et al. 2016 for a technical description). Steps 2 and 3 are updated for each sampling and assessment scenario.

3.6 Sampling scenarios

Three main population scenarios were developped for a south Pacific albacore population assumed to span the Western and Central Pacific Ocean (WCPO) based on each of the stock

assessments (2018, 2021 and 2024). Annual sample sizes for juveniles and adults were trialled over three and four years sampling programs, varying total annual samples from 10,000 to 40,000. The default sampling program assumed even sampling of adults and juveniles, with alternative sampling scenarios varying the total proportion of juveniles in the sample from 0.1 to 0.9. Lastly, design numbers were updated for a population assumed to span both the WCPO and the Eastern Pacific Ocean (EPO) based on the scenarios using the 2021 and 2024 assessments, assuming even sampling of adults and juveniles.

4 Results

Annual samples required to achieve a CV of 15% or less for the recent adult biomass differed depending on the assumed population state as defined by the stock assessment scenario are shown for three and four year sampling programs (Figure 2). For the stock assessment which predicted the smallest population numbers (2021; Figure 1), 36,000 samples were required over three years (corresponding to 6,000 annual samples each of adults and juveniles). For the 2018 stock assessment scenario (predicting higher population numbers), the total annual samples over three years is 60,000 (corresponding to 10,000 annual samples each of adults and juveniles). For the 2024 stock assessment scenario, which predicts the highest population numbers overall, the total annual samples required would be 84,000. CVs on total mortality were high (above 15%) for most sampling scenarios considered (Figure 2, right-hand panel). When the sampling program is extended to four years, improved CVs are achieved for all scenarios under lower annual sampling regimes.

The trends in the number of kin pairs detected matched those in the predicted adult biomass CVs, that is, within each population scenario, a higher number of detected pairs resulted in a lower adult biomass CV (Figure 3), and more pairs are detected when the sampling programs are extented from three to four years. There was less relative improvement in the adult biomass CV achieved past approximately 100 pairs of each kin type.

The kin numbers shown in Figure 3 are expected (average) predictions for each scenario, but in practice the actual number might differ due to natural variability (Figure 4). Despite this, early genotyping of samples collected to date could provide some insights to differentiate between the most likely population scenarios, especially between the 2024 population scenario vs. the 2018 and 2021 population scenarios. This is because a much smaller number of kin pairs would be detected after two years under the population scenario corresponding to the 2024 stock assessment, even when accounting for natural variability. The 2018 and 2021 population scenarios would be harder to differentiate from each other after two years of sampling only.

CVs achieved for adult biomass are robust to some variation in the proportion of adults and juveniles in the annual samples ($\pm$10%, Figure 5). In general, increasing juvenile prevalence in the samples did not overtly impact the adult biomass CV.

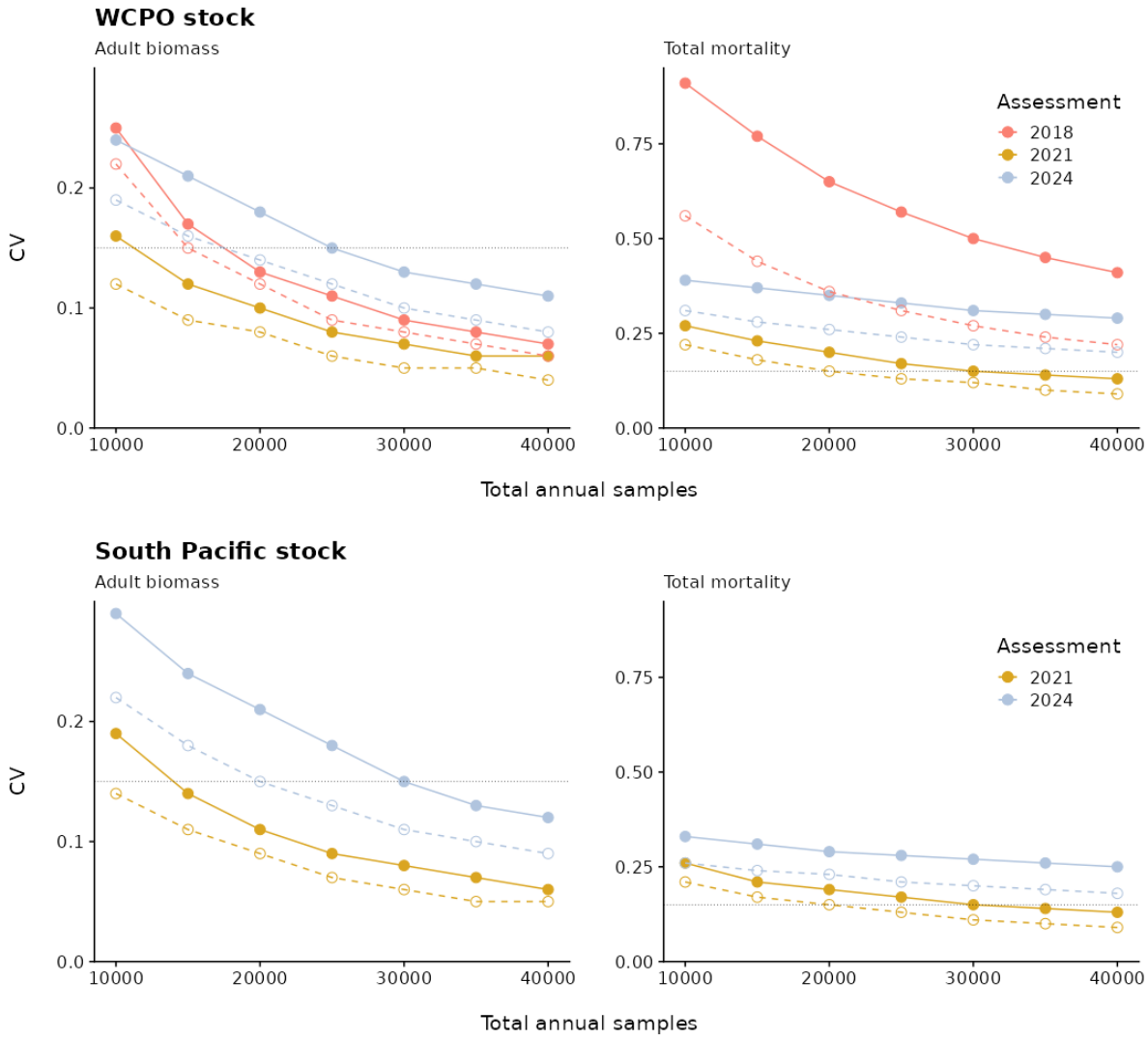

Figure 2: Estimated CVs for adult biomass (left; individuals four years and older) and total mortality (right) under sample sizes evenly spread over three (line) and four (dashed line) years, and by assessment scenario (colours). Note these assume equal numbers of adults and juvenile samples. The top row shows the sampling scenarios assuming a population contained in the WCPO; the bottom row shows the sampling scenarios assuming a population spanning the south Pacific. The dotted hortizontal line shows a potential target CV of 0.15 for reference.

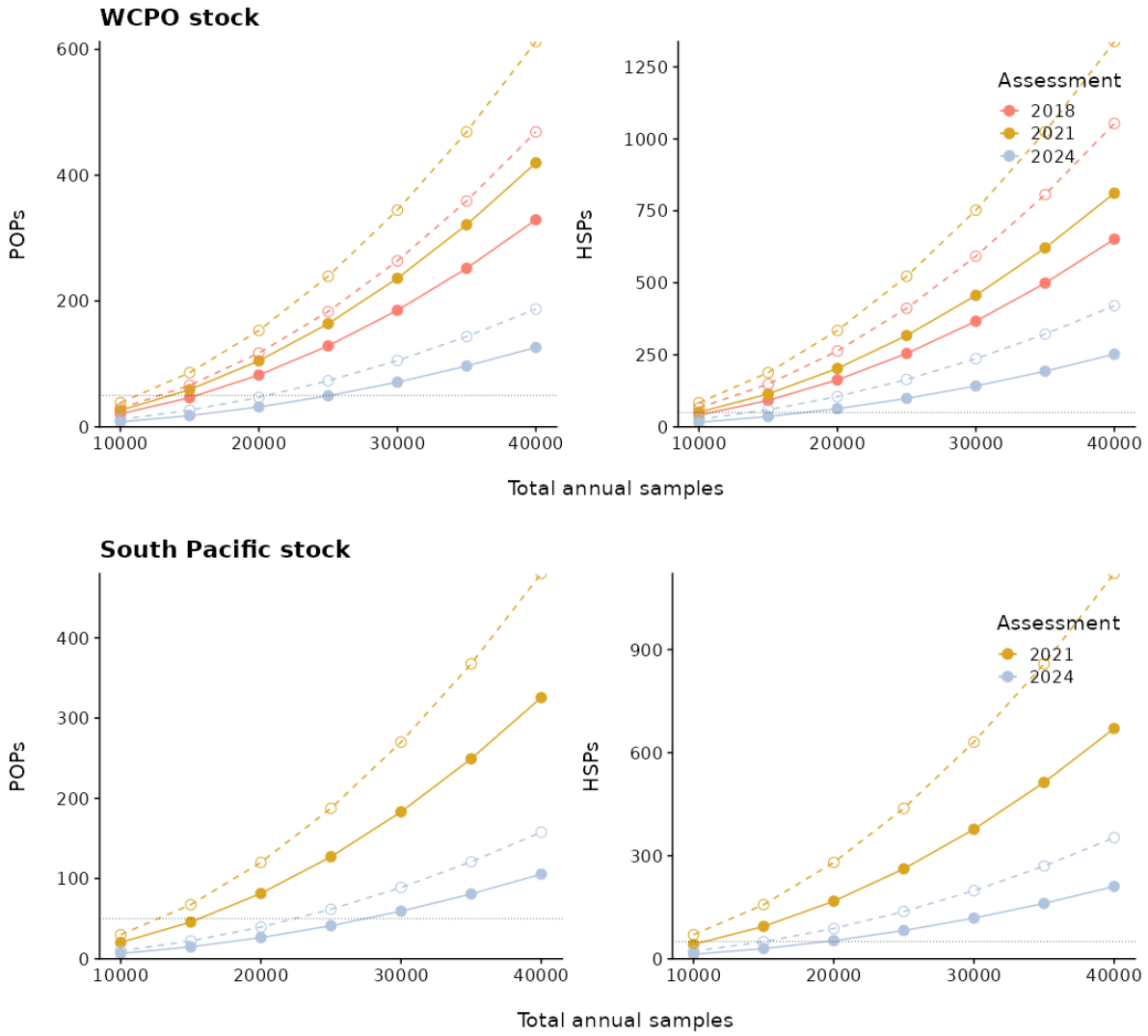

Figure 3: Estimated number of parent-offspring (left; POPs) and half-sibling (right; HSPs) pairs observed under sample sizes evenly spread over three (line) and four (dashed line) years, and by assessment scenario (colours). Note these assume equal numbers of adults and juvenile samples. The top row shows the sampling scenarios assuming a population contained in the WCPO; the bottom row shows the sampling scenarios assuming a population spanning the south Pacific. The dotted horizontal line shows the rule-of-thumb target of 50 for reference.

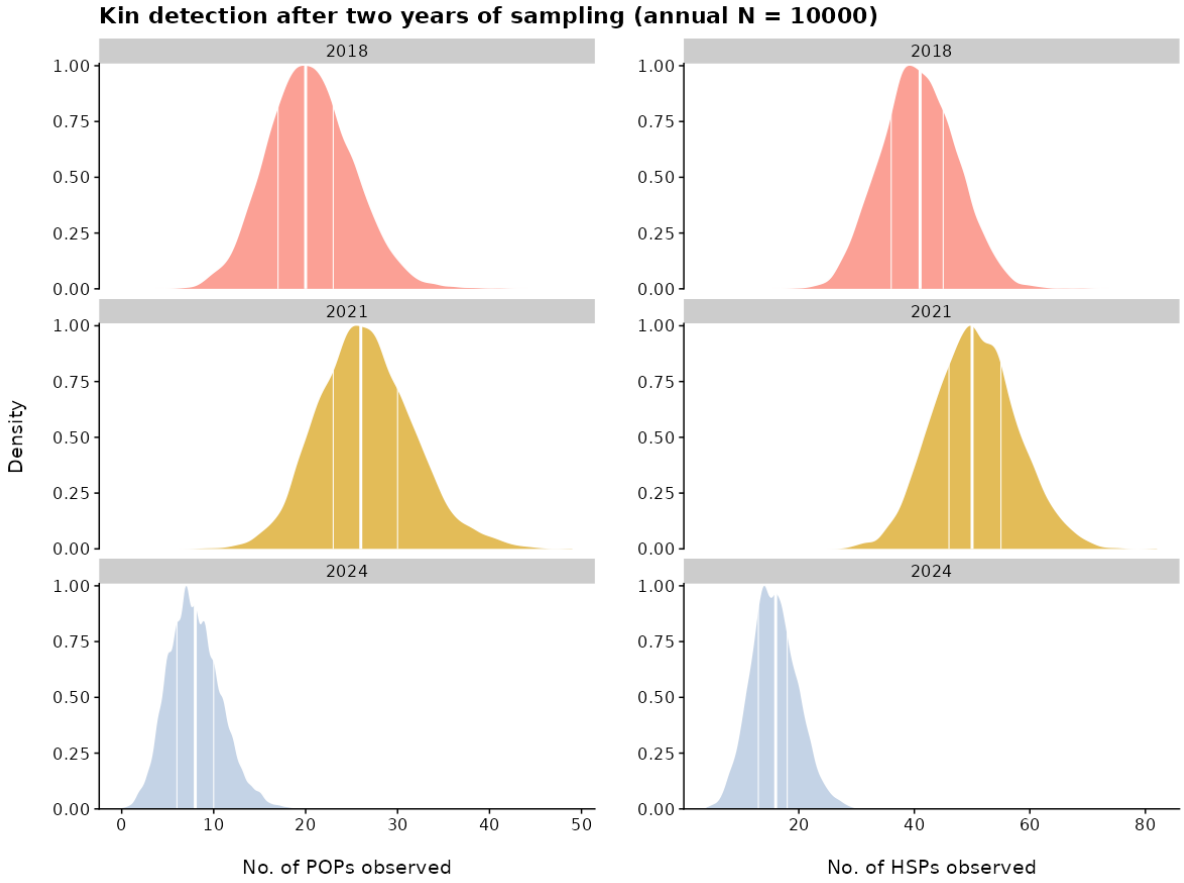

Figure 4: Example of the numbers of kin pairs that could be detected if early genotyping of the first two years of sampling was undertaken (left panel, POPs; right panel, HSPs), assuming 10,000 total annual samples distributed evenly across adults and juveniles and a WCPO-spanning stock. The distributions are shown for each population scenario (by colours). The vertical lines show the 25th, 50th and 75th quantiles of each distribution, corresponding to the probability of observing the associated number of kin pairs under that population scenario.

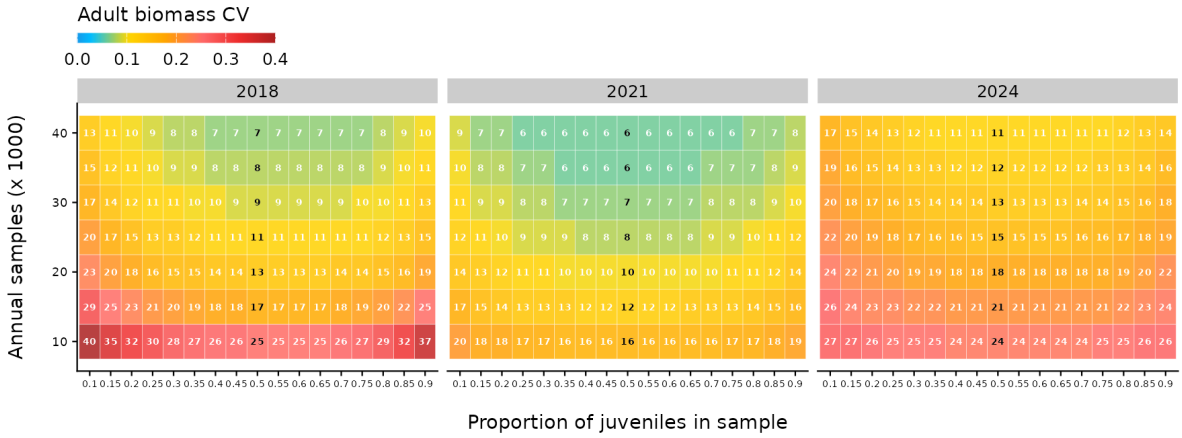

Figure 5: Coefficients of variation (CVs) for adult biomass under two axes of sampling scenarios: increasing proportion of juveniles in the sample (X-axis) and annual number of samples (Y-axis). Numbers in bold highlight the scenarios with equal proportion of adults and juveniles, with the size of the CV depicted by the coloured gradient.

5 Discussion

The sampling design prescribes a range of optimal sample sizes over three years, from 36,000 to 84,000 depending on the population scenario used, and assuming a stock spanning the WCPO. This directly results from changes in some of the methods used in recent stock assessments of south Pacific albacore, which impacted assumptions of population numbers used to drive the sampling design. The increase in the overall sampling number also resulted from the correction to the data inputs used in the initial design estimates.

The wide range in sampling numbers might appear at first impractical for planning. However, the population scenario requiring the least samples is based on the 2021 stock assessment, and concerns were raised about unreasonably low estimates for its predicted recent recruitments (*sensu* the 2016 'big dip' in recruitment, see Scott et al. 2023). As such, it might be cautious to focus on the population scenarios stemming from the 2018 and 2024 stock assessments when planning sampling numbers.

In addition, early genotyping results based on samples collected to date by Project 100c should provide valuable insights as to which population scenario is most plausible, especially with regards to assessing the likelihood of the '2024' population scenario compared to the others.

The wide range of population scenarios estimated from the three most recent stock assessments highlights the appeal of the CKMR approach, which is that, unlike traditional stock assessments, it can provide a reliable estimate of total abundance. As such, the population scenarios used here should only be viewed as informative guidelines when planning the sampling. If more samples were to be collected than needed given the true state of the population (for instance, if 84,000 samples were collected when only 36,000 were needed for informative estimates), the resulting estimates of population metrics would be much more precise than initially aimed for and further improve the quality of the information provided to future integrated stock assessments.

The assumed population numbers driving the design is strongly impacted by assumptions about stock structure, with a wider span (e.g., including the EPO) implying a larger population (and

thus more tissue samples to reliably estimate population size). In addition to other initiatives undertaken under Project 100c to improve our understanding of south Pacific albacore stock structure (e.g., MacDonald et al. 2024), the pattern of kin pair detection resulting from the CKMR sampling program will provide valuable insights as to the likely connectivity of this population across the south Pacific. One advantage of CKMR in that regard is that it provides a signal of connectivity over a single generation, unlike other tools (e.g., population genetics) which can be sensitive to small rates of exchange over longer time frames, and may be of lesser practical use for fisheries managers.

The population model used to inform the sampling design made a number of simplifying assumptions in order to approximate the population dynamics used in the SPA. As such, refinements to aspects of the population model could modify the optimal sampling design structure. However, as the statistical design currently only prescribes the overall scale of sampling required, useful information on south Pacific albacore population scaling and structure will still be gained from the CKMR sampling program even if the assumed population dynamics are wrong. One influential assumption is that the ages of all individuals is known exactly. In practice, age will be estimated from epigenetics, otoliths and/or individual length, which are all imprecise ageing methods with increasing degrees of error, also varying depending on age class. While this will introduce additional uncertainty in the estimated population metrics, allowing for uncertain age in future CKMR population models will increase the number of kin included during model fitting, as same-cohort half-sibling pairs can no longer be excluded (since their age is uncertain, i.e., they might not actually be from the same-cohort). In practice, this inclusion of more kin pairs can compensate to some degree for the added uncertainty from the ageing process. More precise ageing methods should be favoured, but cost by method and varying precision by age class can also be considered when determining the best ageing approach for an individual of a likely age class. For instance, relatively cheap length-based measurements could be favoured for juveniles as these will be a better indicator of age than for adults.

Finally, the sampling numbers produced by the statistical design model assume all samples are of suitable quality for genotyping. In practice, field conditions may result in the collection of degraded and/or contaminated samples. As such, the total number of samples effectively collected by the sampling program needs to account for the potential that some samples will have to be discarded (see SC20-SA-WP-09 for a discussion in the context of Project 100c). As there is some robustness of the sampling design to variability in the proportion of juveniles and adults, increasing samples (within reason) from locations with easier sampling logistics should not overtly impact CKMR modelling outcomes.

Recommendations

SC 20 is invited to:

- consider these updated sampling numbers for the statistical sampling design for south Pacific albacore under Project 100c;
- consider supporting the genotyping of samples collected to date to narrow the range of population scenarios considered from the sampling design;
- consider supporting the investigation into the optimal allocation of ageing methods across ages to minimise ageing costs while improving overall ageing precision.

6 Acknowledgements

Many thanks to John Hampton for his help with data inputs files for the 2024 stock assessment as well as interpretation of earlier design results, Nick Davies for guidance with Multifan-CL output files and software versions, Giulia Anderson for her pointers with regards to genetics and general sampling considerations, and the SPC Pre-Assessment workshop (held in March 2024) who provided early feedback when planning these analyses.

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Appendix 2: SC20/SA-IP-04

SCIENTIFIC COMMITTEE
TWENTIETH REGULAR SESSION

Manila, Philippines
14 – 21 August 2024

Spatial Structure and Regional Connectivity of South Pacific Albacore Tuna in the WCPO and EPO

WCPFC-SC20-2024/SA-IP-04

1 August 2024

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Executive Summary

Responding to a recommendation in the 2021 south Pacific albacore assessment presented to SC17, a two-phase study was initiated to improve knowledge on the population structure of the stock across the western and central Pacific Ocean (WCPO) and eastern Pacific Ocean (EPO).

The study takes a holistic approach, using genetic markers coupled with analyses of otolith shape and otolith microchemistry to explore evidence for population differentiation. The specific aims are to i) help inform decisions on the spatial structure for the 2024 assessment (Teears et al. 2024), along with other supporting analyses (Potts et al. 2024), and ii) help guide sampling strategies and analytical pipelines for the current south Pacific albacore close-kin mark-recapture (CKMR) project (WCPFC Project 100c – SPC-OFP and CSIRO 2024). This Information Paper presents results from Phase 1 of the study, involving a broad-scale comparison between the western WCPO (New Caledonia) and the western EPO (French Polynesia).

Analyses of otolith shape and genetic data from sexually mature individuals captured within the New Caledonian EEZ (n = 55) and the French Polynesian EEZ (n = 55) during November 2022 both support the existence of population differentiation between the two sampling locations. Furthermore, genetic data from an additional 38 individuals captured from New Caledonian waters in June 2022 indicate seasonal stability in the New Caledonian population genomic signature over at least a five-month period from June through November (2022). Thus, seasonal variation cannot explain the genetic differentiation observed between fish collected from New Caledonia and French Polynesia.

These results align with movement rate estimates from SEAPODYM used in the 2024 assessment, and together with other lines of evidence, lend support to the 2-region spatial structure adopted this year. That said, questions remain around the precise location of the longitudinal division in the south Pacific albacore stock; specifically, if this lies within the WCFPC-CA 'overlap' region, now part of sub-regions 1D and 1F, or further west or east? And does this division persist latitudinally?

To address these questions, we recommend follow-up work be undertaken on south Pacific albacore population structure. This work could include completion of Phase 1 analyses and refinement of a Phase 2 design that involves finer-scale sampling across the WCPO and further east into the EPO.

We invite SC20 to:

- Note the Phase 1 results presented in this paper.
- Support the 2024 PAW recommendation for follow-up studies of south Pacific albacore population structure, including completion of the otolith microchemistry component of Phase 1 and refinement of a Phase 2 design, that:
    - i) incorporates finer-scale, structured sampling across the WCPO and further east in the EPO;
        - We note that PAW 2024 highlighted the opportunity for EPO sampling by members that operate vessels in that jurisdiction.
        - We invite SC20 to encourage those members to participate in the necessary sample collection, as well as request SPC-OFP to liaise with the IATTC to enable opportunities for collaborative sample collection.
    - ii) combines empirical and modelled data from a variety of sources where available; and
    - iii) explores intrinsic and environmental mechanisms that might give rise to the observed population structure.

- Recognise the value of multiple lines of evidence, as presented here, to:
    i) help inform decisions on spatial structure in tuna stock assessments (sensu Hamer al. 2023); and
    ii) help inform CKMR sampling designs and analytical pipelines for WCPFC Project 100c.

Background

The representation of spatial structure in stock assessment models can have a strong influence on model outputs and subsequent management advice (Hilborn et al. 2003; Cadrin 2020, 2023). Theory, empirical examples and simulation testing indicate that correctly specifying spatial processes can improve model accuracy (Porch et al. 1998; Punt 2019), help align a model's regional boundaries with biology (Cadrin et al. 2014), improve forecasts on how stocks might respond to various management and environmental change scenarios (e.g. Bell et al. 2018) and reduce socio-economic risks associated with overharvesting or eroded spatial population structure (Ciannelli et al. 2013).

Ideally, all available data on the biological and fishery-related factors relevant to a particular species or stock are gathered and contribute to decisions on the preferred spatial structure for a given assessment (see Moore et al. 2020a, b; Hamer et al. 2023). These data can come from a variety of sources informative at different scales. For example, genetic and genomic data can offer inference on population structure at evolutionary timescales (e.g. Grewe et al. 2015; Pecararo et al. 2018; Bravington et al. 2016, 2021). Tag-recapture data (Williams et al. 2018), otoliths (Macdonald et al. 2013; Duncan et al. 2018; Artetxe-Arrate et al. 2021), muscle stable isotopes (Lorrain et al. 2020), parasites (Moore et al., 2019), meristics, fatty acids and morphometrics (e.g. methods based on length frequency analysis) (Lennert-Cody et al. 2010; Xu et al. 2023; Potts et al. 2024) act at the scale of a fish's lifetime and can contribute to continued advancements in population dynamics and simulation-based models (e.g. SEAPODYM – Senina et al. 2020; Ikamoana – Scutt Phillips et al. 2018). Fishery catch data can provide an intra- and inter-generational perspective (Glaser et al. 2011, 2014; Hamer et al. 2023). Combining inference from multiple data types is seen as good practice to uncover population structure in marine fishes (e.g. Brophy et al. 2020; Taillebois et al. 2021) and this would seem a prudent path towards improving estimates of movement and mixing rates for tropical tunas (Moore et al. 2020b). This information could then be used to guide the selection of candidate spatial structures for the assessment model (Hamer et al. 2023).

In the case of south Pacific albacore (*Thunnus alalunga*), while the limited tagging data available do highlight individuals' capacity to undertake long-range latitudinal and longitudinal movements across the south Pacific (Williams et al. 2015, Figure 2 in Castillo-Jordán et al. 2021), analyses of 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) are indicative of population differentiation between the western and easternmost regions of the WCPFC Convention Area (WCPFC-CA) and between the western and central Pacific Ocean (WCPO) and eastern Pacific Ocean (EPO). These results are supported by the latest SEAPODYM solutions for south Pacific albacore that estimate limited exchange of individuals between the WCPO and the EPO (see Senina et al. 2020; SHOU, ANCORS and SPC 2024; Teears et al. 2024). That said, most empirical studies to date have been constrained by a lack of spatial and temporal resolution and/or structured sampling. And, as highlighted in the review by Moore et al. (2020a), substantial uncertainty remains around the scale of longitudinal movements of south Pacific albacore within the WCPO, and the degree of connectivity between WCPO and EPO populations.

This uncertainty around longitudinal movement dynamics led to recommendations from the 2018 pre-assessment workshop (PAW) to investigate a new spatial structure for the 2018 south Pacific albacore stock assessment to simplify the previous model while retaining biological realism (Pilling and Brouwer 2018). Consequently, the 8-region structure spanning the area of the WCPFC-CA south of the equator used in the 2015 assessment (Harley et al. 2015) was simplified to a 5-region structure in 2018 (see Tremblay-Boyer et al. 2018a, b). The 2021 stock assessment presented to SC17 (Castillo-Jordán et al.

2021) made a further simplification to a 3-region structure with no longitudinal breaks within the WCPFC-CA and represented the first attempt at a fully spatially structured assessment for south Pacific albacore spanning both the WCPO and EPO. It noted:

"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 initiated to better define the population structure of south Pacific albacore across the WCPO and EPO. The study takes a multidisciplinary approach using population genetics coupled with analyses of otolith morphology and otolith microchemistry, building on previous work that used genetic or otolith-based techniques in isolation (e.g. Williams et al. 2012; Macdonald et al. 2013; Anderson et al. 2019). The specific aims are to i) help inform decisions on the spatial structure for the 2024 Pacific-wide south Pacific albacore assessment (Teears et al. 2024), along with other supporting analyses (Potts et al. 2024), and ii) help guide sampling strategies and analytical pipelines for the south Pacific albacore CKMR project (WCPFC Project 100c - Bravington et al. 2021; SPC-OFP and CSIRO 2024).

This Information Paper briefly outlines the study design and presents results from the first phase of the work.

Study design

The study comprises two phases designed to provide inference at different spatial scales:

Phase 1. Broad-scale comparison

Aims to explore evidence for broad-scale population structure in south Pacific albacore within the WCPFC-CA, specifically between the western WCPO (New Caledonia) and the western EPO (French Polynesia).

Phase 2. Finer-scale comparison

The Phase 2 design is partially contingent upon Phase 1 results. It involves finer-scale sampling across a larger geographic area that covers the core region of south Pacific albacore catch in the WCPO and EPO. The results derived from Phase 2 will help pinpoint where any east-west division lies geographically, as well as adding information to our understanding of latitudinal movement dynamics of potential value for CKMR sampling designs.

Sample collection and analysis for the genetics and otolith shape components of Phase 1 are now complete, and we focus on these aspects in the remainder of the paper.

Methods

Sample collection

During November 2022, SPC staff, working in collaboration with fisheries agencies, fishing companies, and the observer and port sampling programmes in French Polynesia (FP) and New Caledonia (NC) collected muscle tissue samples (for high-throughput genomic analyses) and sagittal otoliths (for ageing and morphological and chemical measurements) from 110 sexually mature albacore tuna (fork length range: 80 to 105 cm) captured in each of the FP (n = 55) and NC (n=55) EEZs, within a one-month time window (Figure 1). This sampling protocol was designed to minimise the potential for i) ontogenetic variation in otolith morphology and/or chemical composition to confound any spatial variation that may be present, and ii) confounding temporal variation in genetic and otolith markers with any spatial variation that may be present. An additional 38 muscle tissue samples were collected in New Caledonian waters in June 2022 (Figure 1). We included these in our genetic sequencing efforts to provide an out-group during analyses and to assess seasonal effects on the genetics results.

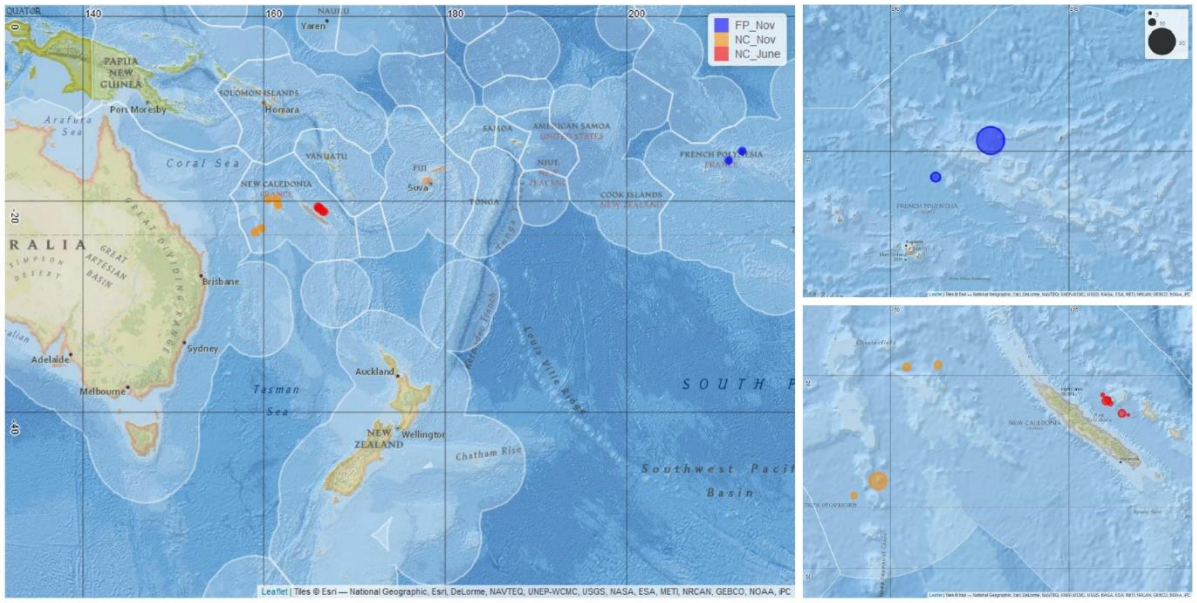

Figure 1. Capture locations for south Pacific albacore in French Polynesian (FP) and New Caledonian (NC) EEZs during June and November 2022. Enlargements of the sampling areas in FP (top right) and NC (bottom right) waters are shown, with circle size in these maps scaled to reflect the sample size per capture location (see black circles at top right for reference).

Muscle tissue samples from fish captured in New Caledonian waters were collected at sea by fisheries observers aboard longline vessels. Fish were sampled on the dorsal musculature just anterior and ventral to the first dorsal spine immediately following capture, using a 3 mm diameter, single-use, medical grade biopsy punch tool (Robbins Instruments). This produced a ~3 × 5 mm tissue sample per fish. Tissue samples were expelled into a sterile 2-ml vial of RNA*later* and stored at −4°C or colder. Each sampled fish's head was then removed and tagged with a cable tie containing a unique ID number before being stored at -20°C or colder for the remainder of the trip. This allowed the tracking of individual fish from vessel to port in Noumea, where the otoliths were extracted, cleaned of adhering tissue and stored dry in labelled vials. In French Polynesia, all fish were sampled in port in Papeete, Tahiti, immediately following unloading from longline vessels. Muscle tissue collection and storage followed the same procedures as for New Caledonia. Otoliths were removed using the drilling method

(https://youtu.be/jbyp_V6C1C0?si=Khfn9Hjs5wjb0tq4) and were cleaned and stored as described above.

Otolith shape analysis

Otolith shape analysis is a well-established approach to uncovering patterns of connectivity and structure in fish populations and has been applied successfully on albacore in the northeast Atlantic (Duncan et al. 2018). We refer readers to Campana and Casselman (1993) and Vignon (2015) for reviews of the mechanisms but note that otolith morphology can be influenced by genetics Cardinale et al. 2004; Vignon and Morat 2010), the environmental conditions experienced throughout life (Hüssy 2008; Berg et al. 2018) and intrinsic processes like somatic growth and feeding history (Hüssy 2008; Denechaud et al. 2020). Several methods have evolved to describe otolith shape. Outline analysis quantifies the boundary shape of the otolith which can be viewed as the lifetime manifestation of the processes mentioned above. We used outline analysis here, requiring only a high-resolution image of each otolith in order to make statistical comparisons of the outlines.

We selected one undamaged otolith per fish (either left or right), photographed the whole otolith under reflected light, sulcus facing down, and measured its mass to the nearest 100 µg. The otolith was then sectioned transversely through the primordium and the fish's age estimated by counting annual growth increments on the resulting thin section. This resulted in age estimates spanning 3 to 11 years across all sampled individuals. Next, we selected fish aged between 4 and 8 years (NC: n = 31; FP: n = 35) as these age classes were the best represented at both sampling locations. Otolith images were first rotated to a standard position, rostrum facing left, and data collected on each otolith's dimensions and outline using the 'shapeR' package (Libungan and Pálsson 2015) in R version 4.3.1 (R Core Team 2023). We measured the maximum length, maximum width, area and perimeter of each otolith, hereafter referred to as 'shape measurements', and detected the outline using the 'detect.outline' function (Claude 2008). To remove size-induced bias, otolith area was normalized to be equal to 1 in all otoliths. We then acquired 64 independent Wavelet coefficients to describe the otolith's outline by conducting a discrete Wavelet transform on equally spaced radii using the 'wavethresh' package (Nason 2022). All shape measurements and Wavelet coefficients were standardised by otolith mass to remove the allometric growth effect on otolith shape. Those showing a significant interaction (at $\alpha = 0.05$) between age class and otolith mass or between sampling location and otolith mass based on analysis of covariance (ANCOVA) were omitted automatically. This step left either three or four shape measurements and 57 Wavelet coefficients for analysis.

To visualise otolith shape variation among age classes and between the two sampling locations (i.e. NC and FP) we used boxplots of shape measurements, reconstructions of mean otolith outlines derived from Wavelet coefficients and ordination results from principal component analyses (PCAs) run in the 'vegan' package (Oksanen et al. 2022). We then tested for an effect of estimated fish age on otolith shape within each sampling location using constrained ordination (i.e. canonical analysis of principal coordinates – CAPs) in 'vegan' and applying an ANOVA-like permutation test to assess the significance of constraints (in this case, fish age) using 1000 permutations. Next, we tested for an effect of sampling location on otolith shape, again using CAP and applying the same permutation test. Finally, we assessed classification accuracy of individuals to their sampling locations in FP and NC based on otolith shape using discriminant analyses of principal components (DAPC) in the 'adegenet' package (Jombart 2008) and random forest (RF) classifiers built in the 'randomForest' package (Liaw and Wiener 2002). The RF hyperparameters were optimised during preliminary tuning, including the 'ntree' argument which we set to 500 across all models. The PCA, CAP, DAPC and RF analyses were run on three different datasets: 1) shape measurements alone, 2) Wavelet coefficients alone and 3) shape measurements

and Wavelet coefficients combined. The analytical pipeline is summarised in Figure 2 and full R code and data to rerun the analysis is available on request.

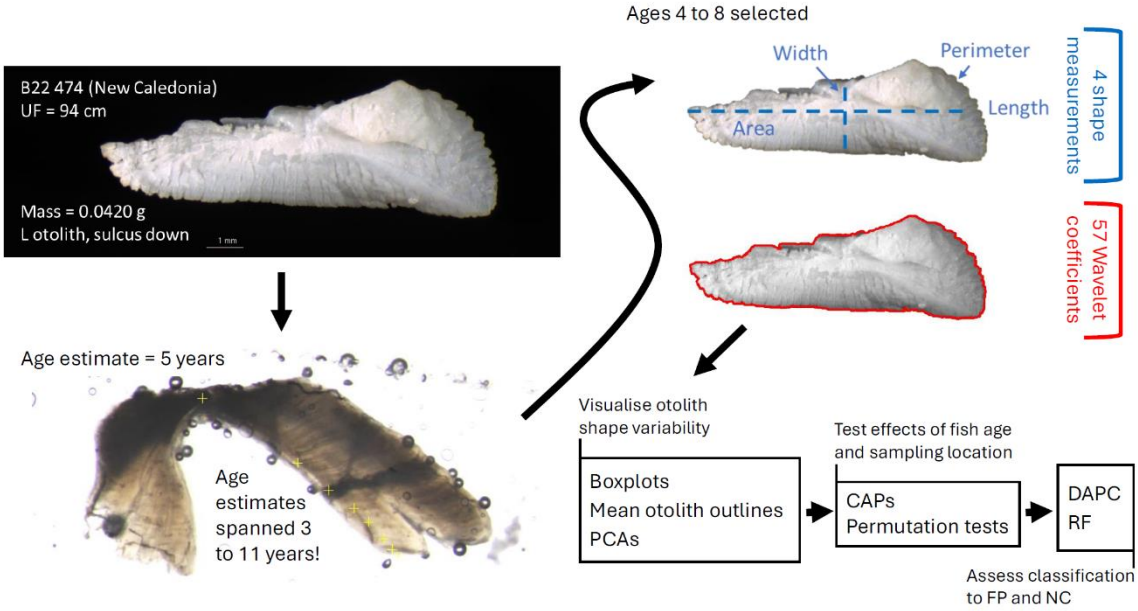

Figure 2. Analytical pipeline for otolith preparation, age estimation and shape analysis. Otolith B22 474 from a 94 cm fork length (UF) south Pacific albacore estimated to be 5 years old is shown for illustration.

Genetic analysis

Genetic samples were sequenced using high-throughput DArTseq protocols by Diversity Arrays Technology, which initially identified over 121,000 loci. The raw dataset was subjected to multiple cycles of quality filters. A fully technical description of the filtering process is available in the Appendix, but in short, we present results from two datasets. The 'primary' dataset includes 6756 datapoints per fish, representing locations from across the genome which were sequenced with very high confidence across all sampled fish. The 'secondary' dataset was further vetted for locations in the genome that carry uncommonly high divergence between FP and NC samples collected in November 2022 (as measured through F_{ST} outlier analyses and disregarding the auxillary sample group during the selection process) to distil patterns of population structure that are otherwise lost in the primary dataset due to the large number of datapoints. The secondary dataset uses 128 datapoints per fish.

The primary dataset was submitted to heterozygosity assessments (which provide population health metrics and are values upon which any other analyses are built) and pairwise comparisons of allele frequencies (which produce single values to quantify degree of difference in genetic signatures between sample groups) using the 'DartRverse' family of packages and StAMPP in R. Various clustering analyses (which employ different assumptions and metrics to reorganise samples into 'logical' groups based on their genetic information) were employed including STRUCTURE, Admixture, and Discriminant Analyses of Principal Components (DAPC, same as is applied to otoliths). Due to its highly selective nature, the secondary dataset was not submitted to heterozygosity assessments, but was submitted to all pairwise and clustering analyses. An additional clustering analysis, StockR, was only applied to the secondary dataset because its algorithm is well designed for marine species but does not include an explicit recommendation for the best number of clusters to report. Again, a full technical description of the genetic analyses can be found in the Appendix.

Results

Otolith shape analysis

Effects of fish age on otolith shape

We detected only subtle differences in otolith shape among age classes for 4- to 8-year-old south Pacific albacore within each sampling location based on standardised shape measurements (Figure A1B) and reconstructions of mean otolith outline per age class from Wavelet coefficients (Figures A1A). These differences were not statistically significant (at $\alpha = 0.05$) for all three datasets (Table 1). The PCA plots in Figure 3C further illustrate the lack of pattern among age classes within sampling locations for all three datasets. Based on these findings. we deemed it appropriate to pool samples from fish aged 4 to 8 within each location for the spatial comparisons between FP and NC.

Table 1. Results from analysis of variance (ANOVA)-like permutation tests of otolith shape variation among age classes within sampling locations, and between sampling locations (when all age classes were pooled within locations). We used 1000 permutations to assess the significance of constraints. d.f. = degrees of freedom; Variance = variance among levels of the constraint; values for Sum of Squares denoted with an *; *F* = pseudo *F*-value (Oksanen et al. 2022).

Tested constraint	Sampling location	Dataset	d.f.	Variance / Sum of squares*	*F*	*p*
Age Residual	FP	Shape measurements	4 30	0.491 2.066	1.782	0.118
	FP	Wavelet coefficients	4 30	5.556* 30.141	1.383	0.102
	FP	Combined	4 30	0.654 2.953	1.662	0.112
	NC	Shape measurements	3 27	0.338 7.081	0.430	0.760
	NC	Wavelet coefficients	3 27	3.479* 31.795	0.702	0.803
	NC	Combined	3 27	0.421 0.1408	0.4655	0.780
Sampling location Residual	-	Shape measurements	1 64	0.096 2.946	2.084	0.126
	-	Wavelet coefficients	1 64	1.833* 65.220	1.798	0.080
	-	Combined	1 64	0.124 3.950	2.011	0.093

Effects of sampling location on otolith shape

With reference to Figure 3, we see that FP and NC otoliths differed in standardised length and perimeter, with NC samples consistently smaller in these dimensions (Figure 3B). The reconstructions of mean otolith outline revealed most of the variation among sampling locations to be between 135° and 170° along the dorsal otolith edge near the rostrum (Figure 3B). The ordination plots (Figure 3C) showed no strong patterns between the two sampling locations along the first two principal components for all three datasets, though the permutation test results (Table 1) do suggest a degree of statistical differentiation between sampling locations driven primarily by variation in Wavelet coefficients. This differentiation is also evident in the density distributions plotted along the first discriminant function of the DAPC run on each dataset (Figure 4).

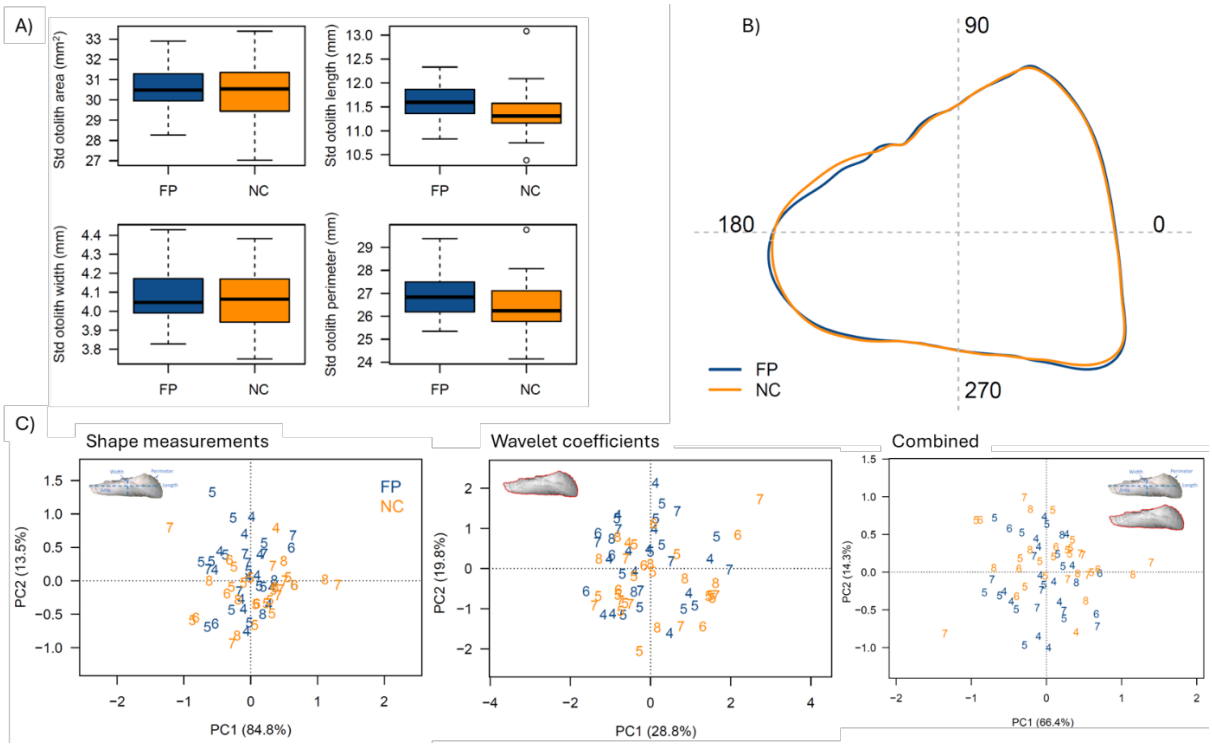

Figure 3. Visualisation of otolith shape variation between sampling locations in FP and NC. A) Box plots summarising the distribution of standardised shape measurements per sampling location. Thick black horizontal lines are the median values for each box and the lower (Q1) and upper (Q3) quartiles (box limits) are shown. Upper whiskers represent the smaller of the maximum value of the variable and Q3 + 1.5 × interquartile range, and lower whiskers the larger of the minimum value of the variable and Q1 - 1.5 × interquartile range. B) Mean reconstructed otolith outline for each sampling location. Numbers refer to angles in degrees. C) Ordination plots describe variation along the first two principal components (PCs) for the three different datasets used. The variance explained by each PC is shown on the axes. The numbers in the ordination plots reflect the age estimate from counts of annual growth increments on a thin section of the same otolith. In all plots, samples from FP are shown in blue, NC in orange.

Regarding classification accuracy, the DAPC on the shape measurements alone showed that, overall, 68.2% of individuals were reassigned correctly to their sampling location. A leave-one-out cross validation (LOO-CV) procedure produced higher misclassification rates (Table 2). The DAPC run on the Wavelet coefficients alone returned a classification success rate of ~60%. Again, LOO-CV classification success was lower. When we combined the shape measurements and Wavelet coefficients, LOO-CV classification success improved to 63.6%. 'Out-of-bag' estimates of classification success on 36.8% of the data held out from training the RF classifiers were similar to the DAPC estimates (Table 2). For both DAPC and RF models, samples from FP were always more accurately classified than NC samples.

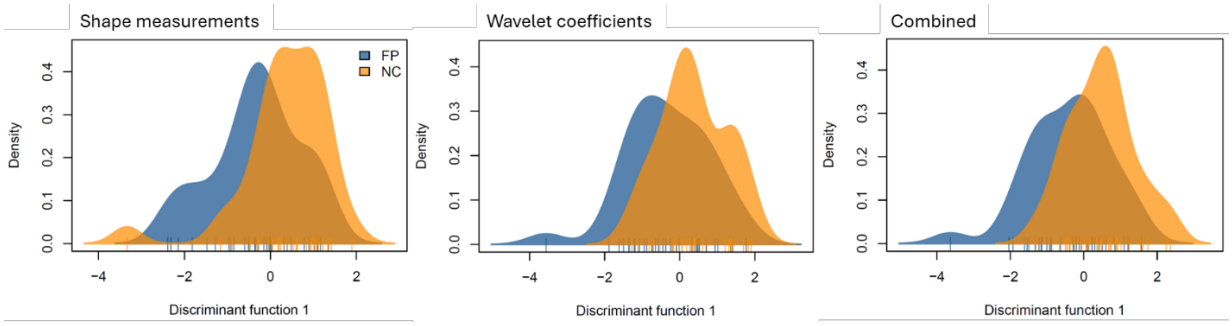

Figure 4. Density distributions for each sampling location along the first discriminant function derived from DAPCs run on each of the three datasets. Samples from FP are shown as blue tick marks on the rug plot, samples from NC are shown as orange tick marks.

Table 2. Percentage of individuals correctly re-assigned to their sampling location based on DAPC and RF classifiers for each of the three datasets. 'all data' = classification based on all data from FP and NC; 'LOO-CV' = leave-one-out cross validation; 'OOB' = out-of-bag; 'FP' = classification based on FP data alone; 'NC' = classification based on NC data alone.

Classification model	Shape measurements	Wavelet coefficients	Combined
DAPC - all data	68	61	67
DAPC - all data LOO-CV	59	55	64
DAPC - FP	71	66	71
DAPC - NC	65	55	61
RF - all data OOB	65	52	62
RF - FP OOB	69	57	71
RF - NC OOB	61	45	52

Genetic analysis

As a reminder, genetic analyses also include an out-group collected in New Caledonia in June 2022 (Figure 1). We therefore label our groups with the added month specificity. Namely, the primary groups are FP_Nov and NC_Nov, and we refer to the additional group as NC_June. We refer to the quality-filtered, genome-wide dataset as 'primary' and the smaller dataset that was filtered for quality and informativeness as 'secondary'.

Heterozygosity-based assessments like observed (H_o), adjusted expected heterozygosity (H_e) and inbreeding coefficient (F_{IS}) provide some general information about the robustness of genetic diversity per sample group and allow for comparisons between groups (lower diversity metrics can imply a smaller or less robust underlying population). Results of these analyses using the primary dataset are provided in Table 3. Pairwise assessment of a related heterozygosity metric, F_{ST}, which quantifies differences in genetic signature on a scale of 0-1, is provided in Table 4. Of key interest, FP_Nov and NC_Nov produced a pairwise F_{ST} value of 0.006 (adjusted p-value = 0) while the two NC samples are not statistically different.

Table 3. Heterozygosity-based metrics with standard deviation in parentheses produced using the primary dataset.

	H_o	H_e	F_{IS}
FP_Nov	0.1321 (0.1196)	0.1505 (0.1343)	0.0986 (0.2238)
NC_June	0.1343 (0.1210)	0.1511 (0.1334)	0.0903 (0.2308)
NC_Nov	0.1302 (0.1176)	0.1491 (0.1330)	0.1028 (0.2267)

Table 4. Pairwise F_{ST} from the primary dataset with metrics provided below the diagonal. Associated p-values adjusted for multiple comparisons using the Benjamini and Yekutieli (2001) method are reported above the diagonal.

	F_{ST}		
	FP_Nov	NC_June	NC_Nov
FP_Nov	---	0	0
NC_June	0.0052	---	0.6892
NC_Nov	0.0058	0.00011	---

We also applied three clustering algorithms to the primary dataset and all concurred on a recommended *k* of 1, suggesting a single population underlies all three sample groups (Figure 5). However, a DAPC identified enough variation to separate the two primary sample groups using 40 principal components (Figure 6).

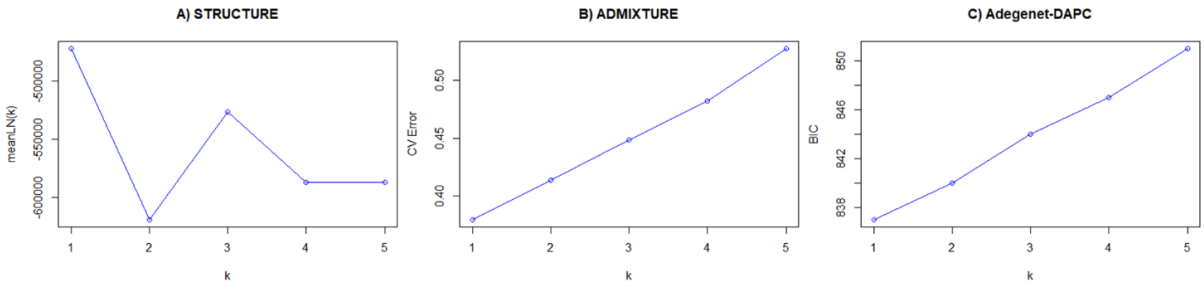

Figure 5. Results from submitting the primary dataset to three clustering programs with different metrics for selecting the most appropriate number of underlying genetic clusters. A) STRUCTURE, where the recommended k is indicated by a plateau in average posterior probability (LnP(K)) and non-convergence suggests *k*=1, B) ADMIXTURE, in which a minimum CV error defines the recommended *k*, and C) DAPC clustering, which recommends the appropriate *k* based on the lowest Bayesian Information Criterion (BIC) value.

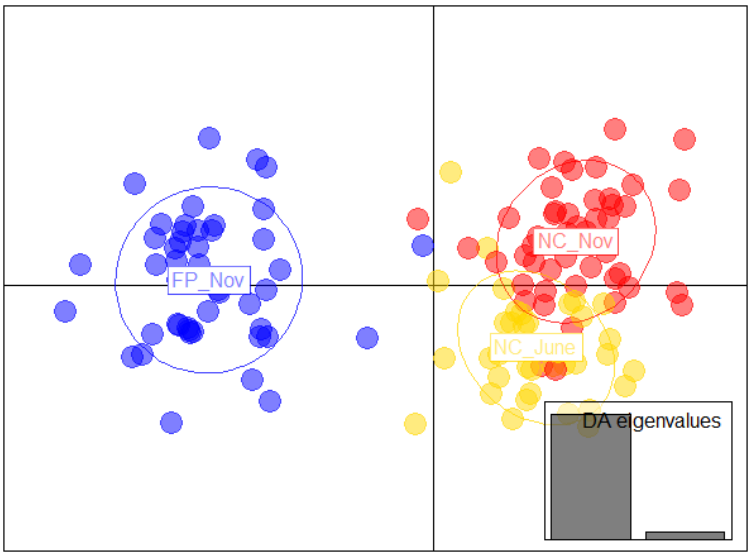

Figure 6. DAPC on the primary genetic dataset using 40 PCs (number of PCs informed by cross-validation) and 2 degrees of freedom

Meanwhile, using the specially selected secondary dataset, heterozygosity assessments were not conducted but we still tested for pairwise F_{ST} (Table 5). Clustering algorithms consistently recommended a *k* of 2 (Figure 7), with subsequent assignment probability analyses (which reassign individuals to the theoretical genetic clusters developed by clustering algorithms) identifying at most three instances of specimens that did not agree with their original geographic groupings (Figure 8, panels A-C). Similarly, a DAPC, which visually groups individuals based on genetic similarity but is not driven by k-clustering, maintains a clear overlap between the two NC sample groups and distinction from the FP samples (Figure 8, panel D). We also note the addition of a result from StockR in Figure 8 (panel C), which produces comparable results using underlying assumptions that are particularly well suited for marine species.

Table 5. Pairwise F_{ST} using the secondary dataset. Values are presented as in Table 4.

	F_{ST}		
	FP_Nov	NC_June	NC_Nov
FP_Nov	---	0	0
NC_June	0.1426	---	0.0029
NC_Nov	0.1581	0.0086	---

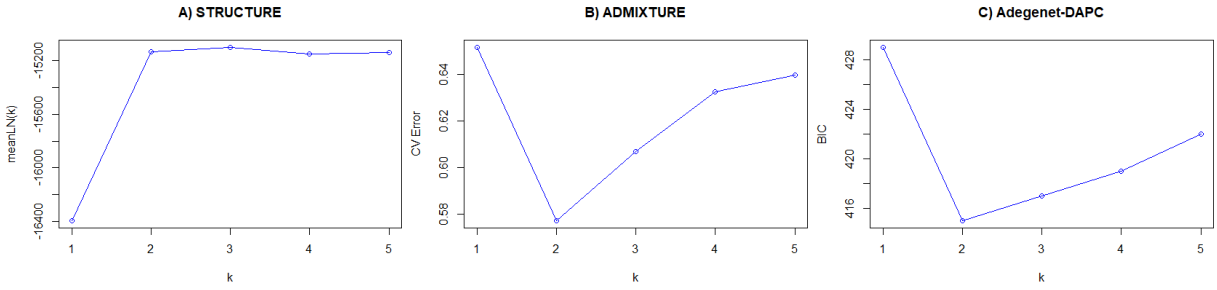

Figure 7. Results from submitting the secondary dataset to three clustering programs. A) STRUCTURE, B) ADMIXTURE, and C) Adegenet-DAPC. Same metrics described as in Figure 5.

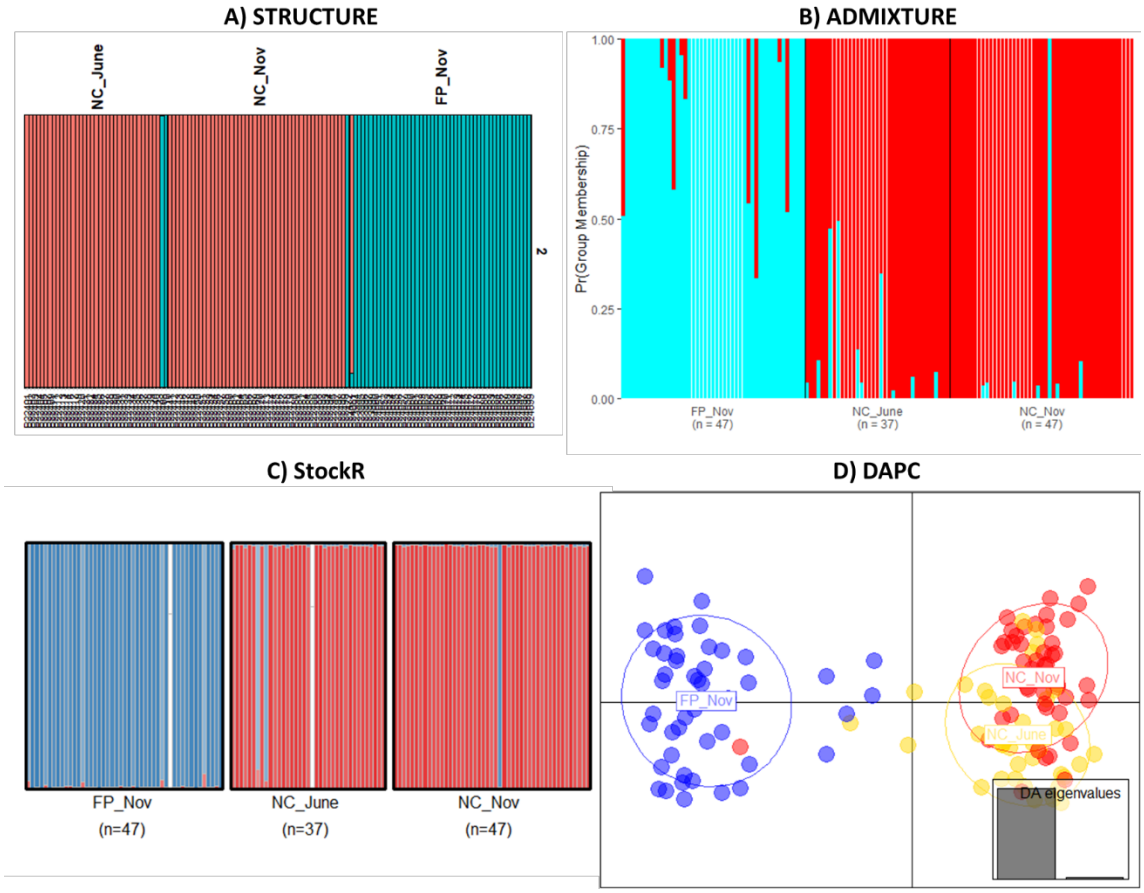

Figure 8. Individual allocations using the secondary dataset by clustering programs given *k* = 2. A) STRUCTURE, where bars represent an individual's probability of assignment to each color-coded cluster, with mixed colored individuals suggesting admixture. B) ADMIXTURE, with same representation as STRUCTURE but constructed using different algorithms. C) StockR, multi-colored bars now represent probability of assignment (not mixed heritage) and colour intensity reflects confidence of assignment. D) DAPC using 10 principal components and 2 discriminant functions.

Discussion

The results from Phase 1 of the study provide strong evidence for the existence of broad-scale population structure in south Pacific albacore between the western WCPO (New Caledonia) and the western EPO (French Polynesia). Moreover, the stability detected in the New Caledonian population genomic signature between June and November 2022 highlights that seasonal variation cannot explain the spatial differentiation we observed between fish collected from New Caledonia and French Polynesia. While acknowledging the small sample sizes available for our comparisons, the agreement observed between the otolith shape and genetic results is notable, particularly as these data types provide inference at different temporal scales and are underpinned by different mechanisms. Taken together, our findings support previous interpretations of the extent of longitudinal structure present within the south Pacific albacore stock based upon analyses of genetic and otolith microchemistry data in isolation (e.g. Montes et al. 2012; Anderson et al. 2019; Macdonald et al. 2013), growth variation and reproductive development (Williams et al. 2012; Farley et al. 2013, 2021) and modelled movement estimates (Senina et al. 2020; SHOU, ANCORS and SPC 2024; Teears et al. 2024).

The Phase 1 sampling protocol – targeting mature south Pacific albacore of 80 cm fork length or larger within the same one-month window in both sampling locations – aimed to minimise potential bias related to ontogenetic and/or temporal variation in otolith and genetic markers that could confound our interpretation of geographic variation in these markers between FP and NC. Despite achieving these sampling objectives, the large range of ages (i.e. 3 to 11 years) estimated for our sampled fish necessitated a test for age effects within each sampling location as a first step in the otolith shape analysis. We detected only subtle differences in mean otolith outlines among age classes for 4- to 8-year-old individuals when shape descriptors were standardised by otolith mass[6], allowing data to be pooled across age classes within sampling locations. Importantly, subsequent spatial comparisons using permutation tests, DAPC and RF classifiers on the range of shape descriptors analysed were all in agreement, indicating a degree of differentiation between FP and NC samples.

Otolith outlines can be viewed as life-time representations of both the intrinsic (e.g. genetics, somatic growth rate, fish size, feeding history) and environmental factors (e.g. temperature, depth, prey availability) experienced by individual fish (Cardinale et al. 2004; Hüssy 2008; Vignon and Morat 2010; Denechaud et al. 2020). While distinguishing the relative contribution of these factors remains challenging (Vignon 2015), it is increasingly recognised that allometry is a key determinant, and that the experienced environment can act indirectly – via dictating patterns of growth increment formation throughout life which in turn strongly influence otolith morphology (Campana and Casselman 1993; Cardinale et al. 2004; Vignon 2015; Denechaud et al. 2020).

With these points in mind, we propose three scenarios that could potentially give rise to the variation we observed in shape descriptors between FP and NC and that could be further explored in a Phase 2 of the study. The first has an environmental basis – juveniles from a single spawning stock following divergent migration pathways in the WCPO and EPO, traversing environment gradients different enough to induce some morphological differences in adult otolith outline. Yet given the genetics results presented herein and in previous work (Takagi et al. 2001; Montes et al. 2012; Anderson et al. 2019), the single spawning stock hypothesis for south Pacific albacore seems highly unlikely (is not genetically possible). A second and more likely explanation involves two (or more) geographically distinct spawning groups with very limited mixing between reproductive spawning aggregations that are exposed to moderately different environmental forces across the lifetimes of group members, as enforced by local and/or regional oceanography. Third, assuming the existence of two (or more) spawning groups, the spatial differences we see could have a genetic basis (Cardinale et al. 2004; Berg et al. 2018) or arise through genetic differences mediated by differences in environmental exposure (Vignon and Morat 2010).

Seeking evidence for each of these scenarios requires additional data. In particular, further work is required to understand oceanographic processes in the south Pacific and north Pacific Tropical Gyres, which bring juveniles to the New Zealand troll fishery. Moreover, there is a need to resolve the ecological and evolutionary connections between juveniles and adults in the EPO and mixing rates between eastern EPO and WCPO populations, noting that samples from juveniles in the EPO have historically been difficult to obtain due to lower fishing activity in that region (SHOU, ANCORS and SPC 2024). Otolith microchemistry data may offer useful insights, generating a time-stamped, individual-

[6] Standardisation by fish length or otolith length is commonly used to account for the allometric growth effect on otolith shape (see Cardinale et al. 2004; Libungan et al. 2015). Our decision to use otolith mass instead was driven by i) the tight relationship observed between fork length and otolith mass for our samples and ii) higher confidence in the accuracy of the otolith weight measurements compared with some uncertainties around some fork length measurements recorded in the field. We note that our results were insensitive to using either fish length or otolith mass for standardisation.

level record of environmental and physiological experience. This approach has proved informative in past studies of movement and mixing in south Pacific albacore (Macdonald et al. 2013). Importantly, it complements other empirical and simulation-based approaches (Sakamoto et al. 2019; Brophy et al. 2020; Taillebois et al. 2022), letting us track connections among individuals and populations at specific life history phases (e.g. hatching, spawning). The otolith microchemistry component of Phase 1 is yet to be completed, and we recommend pursuing this, as well as follow-up studies like that outlined in Phase 2, which extends the Phase 1 design through finer-scale sampling across the WCPO and into the eastern EPO. Following these recommendations would have the dual benefit of informing on the mechanisms driving population structure across the region and guiding aspects of the CKMR design work.

The Phase 1 genetic results come to a similar conclusion as the otolith shape results using different biological mechanisms and timescales. Namely, the data supports the presence of biologically important population structure between FP and NC. Although some of our evidence may seem to contradict this conclusion (namely clustering algorithms that support a single underlying population using the primary dataset) it is a matter of terminology—the presence of structure being different from the presence of discrete populations. The 'primary' dataset, which captures genome-wide differentiation between sample groups, still accurately separates samples by location via DAPC and produces statistically significant differences in group genetic signature via pairwise F_{ST}. Furthermore, the comparison of the two NC sample groups in both analyses quantifies the degree of stochastic differentiation one could expect when sampling the same (sub)population twice, which is a magnitude lower than FP-NC comparisons and not statistically significant.

We also present results from the 'secondary' genetic dataset, which is a distillation of F_{ST} outlier datapoints. We emphasise the risk of misrepresenting larger trends when cherry-picking 2% of available datapoints and limit our interpretation to two points. First, the French Polynesian-New Caledonian structure reported by pairwise F_{ST} and DAPC in the primary dataset is further supported by the individual reassignment steps of all clustering algorithms (a step that is uninformative using the primary dataset, given that the recommended number of underlying populations is one). Second, due to the nature of F_{ST} outlier-based selection, there is an increased chance that the parts of the genome involved may experience adaptive pressure, such as from environmental stressors. Identifying environmental drivers of population structure is a very useful step towards describing the larger population dynamics, and the existence of loci showing outlier patterns of allele frequency distribution suggests it would be worthwhile to consider a follow-up study that captures environmental as well as geographic variation.

These results are consistent with the literature. Our primary and secondary datasets are comparable to neutral and potentially adaptive datasets, respectively, in two other papers that have applied Next Generation Sequencing technology to Pacific albacore stocks (Anderson et al 2019, Vaux et al 2021). In both a west-central South Pacific comparison and North-South Pacific comparison, strong patterns of population structure were reported using potentially adaptive datasets, while the signal was much weaker using neutral datasets.

Like the otolith shape dataset, we note that the current genetics datasets are not equipped to propose a complete theory about the state of population structure in the south Pacific or what the exact drivers are. As stated above, we therefore recommend undertaking Phase 2 of the study to more thoroughly explore the correlation of genetic and otolith variation with various environmental forcings. From a genetics standpoint, we would particularly encourage the collection of samples from still further east in the EPO, as a way to capture more divergent environmental conditions that may better clarify drivers of adaptive population structure.

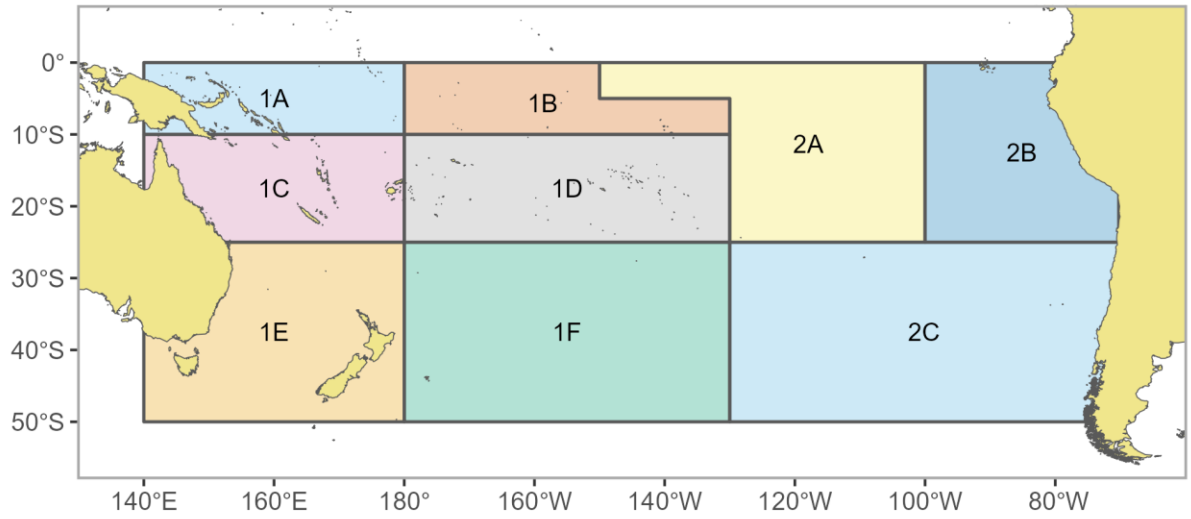

Figure 9. Spatial structure used in the 2024 south Pacific albacore assessment. Numbers 1 and 2 indicate explicit regions in the assessment model. The letters (A, B, etc) indicate sub-regions used for the definition of fisheries (redrawn from Teears et al. 2024).

Do our findings support the 2024 spatial structure?

Some discussion is also warranted on how our results align with the spatial structure chosen for the 2024 south Pacific albacore assessment. The 2024 assessment uses a simplification of the 4-region structure used in the 2021 assessment (Castillo-Jordán et al. 2021) to a 2-region structure with sub-regions defined by fisheries (Figure 9). Region 1 corresponds to the area of the WCPO within the WCPFC-CA from the equator to 50°S, including the 'overlap' region, while region 2 encompasses the same latitudes in the EPO, within the Inter American Tropical Tuna Commission (IATTC) Convention Area, excluding the overlap region (Figure 9).

Movement between regions 1 and 2 is estimated as age and season specific. In the absence of informative tag-recapture data, particularly in relation to longitudinal movement rates, the 2024 PAW proposed the use of the SEAPODYM model to provide information on recruitment distribution and movement rates across life stages (Senina et al. 2020, Hamer 2024 [SC20-2024/SA-IP-01]). This plan was adopted in the 2024 assessment (Teears et al. 2024). In summary, SEAPODYM estimated low movement rates between regions 1 and 2 across all age classes, with negligible influence of season on these estimates. Four additional sensitivities to the movement specification were considered i) zero movement between the WCPO and EPO regions, and ii) lower movement (i.e. movement probabilities half those estimated by SEAPODYM), iii) higher movement (i.e. movement probabilities double those estimated by SEAPODYM) and iv) movement approximating full and instantaneous mixing of the stock. Sensitivities i) and iv) were not considered biologically plausible; however, these were included in the sensitivity runs as extreme lower and upper limits. Overall, the stock assessment results appeared to be robust to the movement assumption and it was deemed unnecessary to consider alternative movement scenarios in the final model ensemble (Teears et al. 2024).

Our empirical findings are in line with the SEAPODYM estimates, noting that the Phase 1 sampling coverage only encompasses the overlap region of the EPO and not further east, whereas SEAPODYM estimates are calculated across the entire assessment domain. Moreover, our findings, in conjunction with other lines of evidence (Williams et al. 2012; Farley et al. 2013, 2021; Takagi et al. 2001; Montes

et al. 2012; Macdonald et al. 2013; Anderson et al. 2019; Potts et al. 2024) largely support the chosen 2-region structure for the 2024 assessment. That said, important questions remain around the precise location of the longitudinal division in the south Pacific stock; specifically, if this lies within the WCFPC-CA overlap region, now part of sub-regions 1D and 1F, or further west or east? And does this division persist latitudinally? A Phase 2 of the study, incorporating finer-scale sampling across a broader region of the WCPO and EPO and integrating other empirical and modelled data where available, would help to answer these questions.

Recommendations

We invite SC20 to:

- Note the Phase 1 results presented in this paper.
- Support the 2024 PAW recommendation for follow-up studies of south Pacific albacore population structure, including completion of the otolith microchemistry component of Phase 1 and refinement of a Phase 2 design, that:
    iv) incorporates finer-scale, structured sampling across the WCPO and further east in the EPO;
        – We note that PAW 2024 highlighted the opportunity for EPO sampling by members that operate vessels in that jurisdiction.
        – We invite SC20 to encourage those members to participate in the necessary sample collection, as well as request SPC-OFP to liaise with the IATTC to enable opportunities for collaborative sample collection.
    v) combines empirical and modelled data from a variety of sources where available; and
    vi) explores intrinsic and environmental mechanisms that might give rise to the observed population structure.
- Recognise the value of multiple lines of evidence, as presented here, to:
    i) help inform decisions on spatial structure in tuna stock assessments (sensu Hamer al. 2023); and
    ii) help inform CKMR sampling designs and analytical pipelines for WCPFC Project 100c.

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Appendix

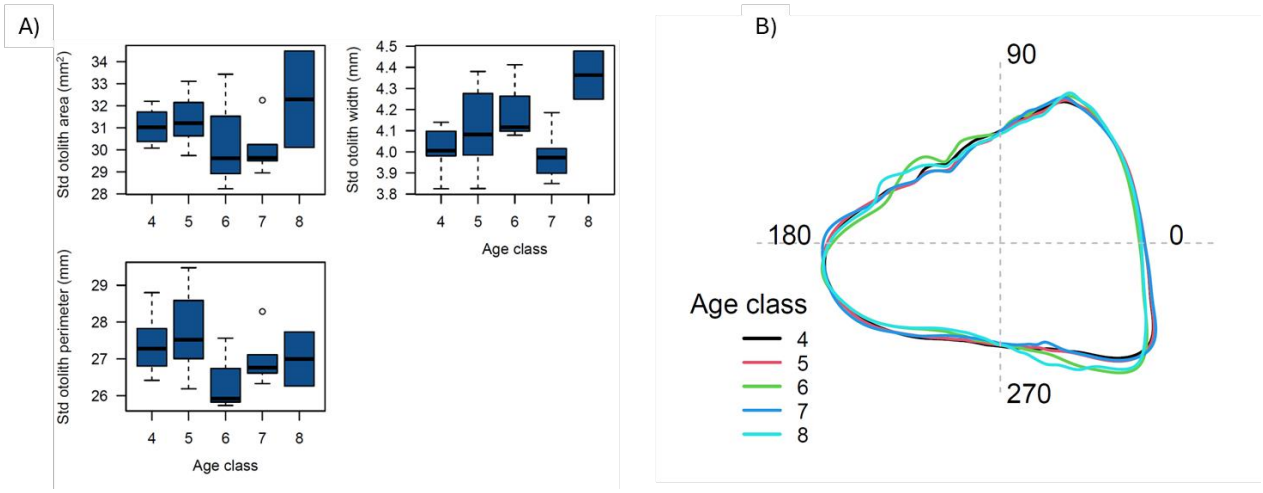

Figure A1. Visualisation of otolith shape variation among age classes in FP. (A) Box plots summarising the distribution of standardised shape measurements per age class. Thick black horizontal lines are the median values for each box and the lower (Q1) and upper (Q3) quartiles (box limits) are shown. Upper whiskers represent the smaller of the maximum value of the variable and Q3 + 1.5 × interquartile range, and lower whiskers the larger of the minimum value of the variable and Q1 - 1.5 × interquartile range. (B) Mean reconstructed otolith outline for each age class. Numbers refer to angles in degrees.

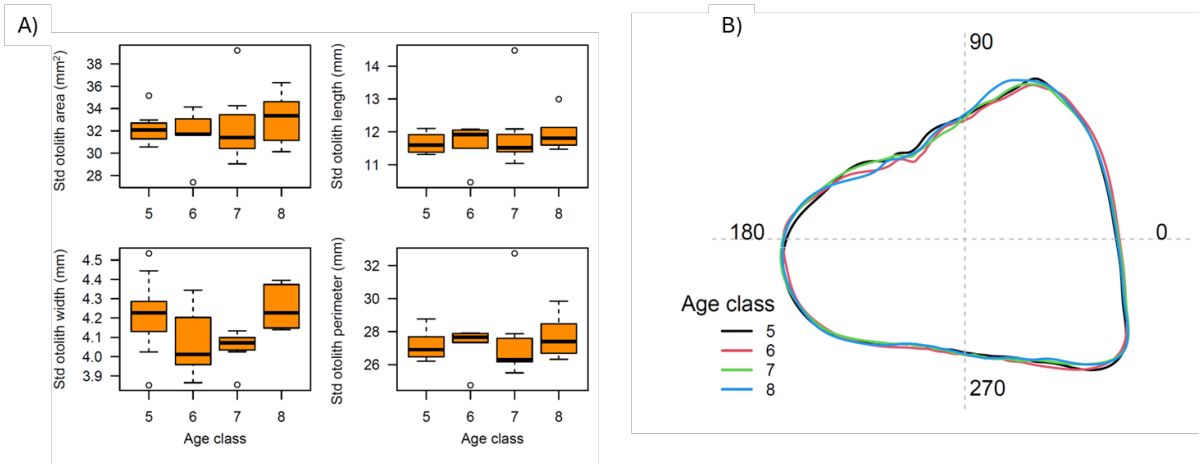

Figure A2. Visualisation of otolith shape variation among age classes in NC. All details for A) and B) as in Figure A1.

DNA extraction and sequencing, conducted by Diversity Arrays Technology

Tissue samples were provided to Diversity Arrays Technology as part of a larger sequencing effort.

Genomic DNA was extracted and processed for reduced representation library construction, sequenced and genotyped 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). Briefly, DNA was extracted in the DArT PL laboratory, presumably with a basic CTAB protocol. Purified DNA then underwent genome complexity reduction with a double restriction digest using methylation-sensitive restriction enzymes, which is a method DArT PL has previously optimised for tuna samples. 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. 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 in order to call genotypes that are study-specific.

Additional quality filtering and development of final datasets

The final dataset provided by DArTech PL included 121105 loci and 131 samples in three sample groups: NC_June (37 samples taken in New Caledonian waters in June 2022), NC_Nov (47 samples from New Caledonia in November 2022) and FP_Nov (47 samples from French Polynesia in November 2022). We proceeded to filter the original dataset twice. Filtering was either done manually or using the DartRverse package in R. Specifics about number of loci filtered at each step in either dataset are available in Table 1, below.

The first, primary dataset removed loci based on the following quality filters thresholds calculated globally across all three sample groups:

- all loci per contig except that with the highest information content
- loci with a call rate < 95%
- with a read depth below 12x or above 100x
- more than 50% heterozygosity
- minor allele count lower than 5x
- deviation from Hardy-Weinberg equilibrium in more than one sample group after adjustment of associated p-values using the (Benjamini and Yekutieli 2001) correction method
- loci in linkage disequilibrium with a correlation $r^2 > 0.2$, also informed by metadata provided by DArTech after an attempt to map loci to an available bluefin tuna reference genome (part of the genotyping pipeline)

A second dataset was created to capture loci that are F_{ST} outliers. To do this, we adjusted our filtering thresholds slightly to include more loci with rare alleles, which are more likely to be flagged in F_{ST} outlier analyses but carry higher risk of being a sequencing error. We simultaneously increased other quality filter thresholds to ensure we did not retain erroneous loci. Furthermore, we only considered samples from FP_Nov and NC_Nov to ensure selected loci were explicitly informative for this spatial

comparison. Once all filtering and selection processes were complete, we re-incorporated the NC_June data for the same loci. Specifically, we filtered based on:

- all loci per contig except that with the highest information content
- loci with a call rate < 95%
- with a read depth below 15x or above 100x
- more than 50% heterozygosity
- minor allele count lower than 3x
- deviation from Hardy-Weinberg equilibrium in more than one sample group after adjustment of associated p-values using the Benjamini & Yekutieli (2001) correction method
- loci in linkage disequilibrium with a correlation $r^2 > 0.2$, also informed by metadata provided by DArTech after an attempt to map loci to an available bluefin tuna reference genome (part of the genotyping pipeline)

The resulting dataset was then submitted to four different programs that detect F_{ST} outlier loci.

- Bayescan (Foll and Gaggiotti, 2008): combines a Dirichlet distribution model with a Bayesian method to estimate each's loci's posterior probability. It is one of the most frequently cited F_{ST} outlier detection programs in the literature (2886 citations at the time of writing).
- HacDivSel, Exterme Outlier Set test (Carvajal-Rodríguez, 2017): uses a two-step G_{ST} outlier test that minimises false positive discovery rate in scenarios of moderate or high migration rates, which makes it particularly relevant to tuna.
- Outflank (Whitlock and Lotterhos, 2015): uses a revised Lewontin-Krakauer model and was specifically designed to be more flexible when defining the neutral distribution of loci for a study. This is again helpful given the uncertain population model of tuna and demonstrated high neutral diversity.
- PCAdapt (Luu et al. 2016): tests for structure by principal component analysis prior to testing for FST outlier loci. It is particularly geared toward identifying local adaptation as opposed to other drivers of adaptation and is consciously designed to handle admixed individuals, which are additional likely scenarios for tuna.

All software programs were run using default settings, apart from specifying a false discovery rate of 5% where applicable. Outflank and PCAdapt also allow modification of the minimum minor allele frequency per loci, which we specified as close to zero as each software allows, in an effort to preserve adaptive structure driven by rare alleles (Linck and Battey, 2019).

Loci were included in the secondary dataset if they were flagged by any of the considered software programs. We also ran analyses using a dataset that only retained loci identified by two or more software programs, but this produced the same trends and suffered from reduced statistical power.

Table A1. loci retained at each filtering step for the two datasets, including FST outlier selection specific to the secondary dataset.

Filter type	Dataset	
	Primary	Secondary
Quality Filters		
Original	121105	121105
Replicates per contig	61705	61705
Call rate	19940	19566
Read depth	15704	14948
Heterozygosity	15627	14865
Minor allele count	6891	6797
Hardy Weinberg equilibrium	6890	6793
Linkage disequilibrium	6756	6730
FST outliers		
Bayescan	NA	23
HacDivSel	NA	74
Outflank	NA	38
PCAdapt	NA	21

While F_{ST} outlier status is often used as a proxy to identify loci that are under adaptive pressure, our study design using only two sample groups makes it difficult to determine what external forces might be associated with any flagged loci. We therefore treat this dataset more as a cherry-picked panel of loci to help distil otherwise very subtle patterns reported using the primary dataset.

Further details about genetic analysis methods

We provide coding details here for reproducing all genetic analyses conducted in programs. They are ordered as they appear in the main text. All processes run in R use v 4.2.2 (R Core Team, 2022).

- Ho, unbiased He (reported as µHe), F_{IS} —calculated using *DartR.base* package v 0.65 in R (Gruber et al. 2018; Mijangos et al. 2022), command 'gl.report.heterozygosity'
- Pairwise F_{ST}—*StAMPP* package v 1.6.3 in R (Pembleton et al. 2013), 'stamppFst' specifying 'nboots=10000'
- Pairwise F_{ST} p values—adjusted for multiple comparisions using *stats* package v 4.5.0 in R (R Core Team, 2022), command 'p.adjust' specifying 'method = 'BY'' for the Benjamini & Yekutieli (2001) correction method
- DAPC—*adegenet* package v 2.1.10 in R (Jombart, 2008; Jombart and Ahmed, 2011), using command 'dapc' and specifying number of principal components and discriminant functions interactively. Number of PC's was informed by cross validation
- DAPC cross validation—*adegenet*, using command 'xvalDapc' on a genind object transformed into a matrix (via R base command 'tab', specifying 'Na.method='mean') and reporting output [6], Number of PCs Achieving Lowest MSE, as the number of PC's to use.
- STRUCTURE—self-contained software package v 2.3 (Pritchard et al. 2000) run in the R environment via package *strataG* v 2.5.01 (Archer et al. 2017), command 'structureRun' specifying 'k.range=1:5, num.k.rep=5, burnin=5000, numreps=50000'
- STRUCTURE k-selection—*StrataG*, command 'evanno' specifying the output of 'structureRun'; appropriate k value selected based on indicators recommended in (Pritchard, Stephens, and Donnelly 2000; Evanno, Regnaut, and Goudet 2005) namely picking the first

value of k to produce a plateau in value meanLnP(K) (the mean log probability of results at a given k value) of and/or a spike in Δk (change in the log probability value between successive k's). Failure of results to conform to expected outputs patterns for either metric was interpreted as support for k=1.

- STRUCTURE visualisation—*DartR.popgen* v 0.3.2 R (Gruber et al. 2018; Mijangos et al. 2022) (wrapping functions from *StrataG*), command 'gl.plot.structure' specifying the output of 'structureRun' and 'K=2' for the LUPS dataset.
- ADMIXTURE—self-contained software v 1.3.0 (Alexander et al. 2009; Zhou et al. 2011), run via command line './admixture' specifying '--cv FILE.bed k|tee logk.out' over sequential k-values = 1-10. Cross-validation values are saved into logk.out files and retrieved to compare and select appropriate k based on lowest CV.
- ADMIXTURE visualisation—First augment the ADMIXTURE output q-file for the selected k value ('outfile.Q.[k]') to include a new column 1, labelled 'orig.pop' and populated with the group name per individual. Then submit to *strataG* in R, command 'structurePlot', specifying 'pop.col=1, prob.col=2, sort.probs=FALSE, horiz=FALSE, type='bar', legend.position="none", col=rainbow(k)).
- DAPC-k means clustering—*adegenet*, using command 'find.clusters' and interactively selecting to keep all available principal components and discriminant functions. Function returns output$Kstat with Bayesian Information Criterion values for k=1-20 and the lowest BIC value used to identify the most appropriate k.
- StockR individual assignment—*StockR* package v 1.0.76 in R (Foster et al. 2018), 'stockSTRUCTURE' run iteratively specifying K=1-5 to fit the data to between 1 and 5 underlying populations, 'stockBOOT' specifying B=500 to test the confidence of individual assignments over 500 bootstraps, and 'plot.stockBOOT.object' specifying CI.width=0.95 to manipulate the scaling of color intensities in returned visuals.

Appreciable differences between clustering programs

As with F_{ST} outlier selection programs, there are an increasing number of software programs that provide genetic clustering recommendations with different sensitivities and underlying assumptions. We provide a few more insights into each of our selected algorithms here. Exact commands are provided in the section above.

- STRUCTURE (Pritchard et al. 2000): is the most cited clustering program in the literature (over 38000 citations of (Pritchard, Stephens, and Donnelly 2000) at the time of writing). It uses a Bayesian approach and Markov Chain Monte Carlo estimation to calculate each individual's probability of assignment to each cluster within a given k. Thanks to the base program's popularity, a number of additional programs have been developed to improve interpretation and readability of the output, and compensate for a number of acknowledged blind spots (which leads to our incorporation of recommendations by (Evanno, Regnaut, and Goudet 2005)). A drawback of the program is its underlying population model assumptions, some of which are not well fitted to a tuna's life history. Regardless, we choose to use the software for the sake of comparison with existing literature.
- ADMIXTURE (Alexander et al. 2009; Zhou et al. 2011): uses the same basic model as STRUCTURE, but with a block relaxation algorithm and maximum likelihood framework to assign individuals to the given number of clusters (k). It is specifically advertised as a follow-up to STRUCTURE that is much faster to run and handles admixed individuals better.
- Adegenet (Jombart, 2008; Jombart and Ahmed, 2011): As part of conducting a DAPC, adegenet also provides a computation-efficient clustering approach that applies *k*-means clustering to a PCA-transformed dataset. In contrast to STRUCTURE, it does not require selection of a population structure model, therefore allowing for much more fluid applications. Although the adegenet package does also provide options to visualise confidence of individual assignment to their group, the software manual (Jombart and

Collins, 2015) warns against using this feature on data re-grouped according to clustering recommendations, as this would only create circular validation. We therefore only present the BIC results of *k*-means clustering and a complete DAPC scatterplot of samples in their *a priori* groups.

- StockR (Foster et al. 2018): first employs *k*-means clustering similar to adegenet, followed by a final classification by EM-algorithm (expectation-maximum) approach and confidence in each individual's assignment measured using Bayesian bootstraps. This is one of the few programs designed explicitly to clarify stock delineations, as opposed to identifying founder populations. As such, it is less prescriptive about the underlying *k*-value, but can reveal potentially relevant substructure at diverse *k*'s. It is therefore only reported for the LUPS dataset, where there is sufficient external evidence to support *k*=2.

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