Identifying biological stocks of Silver Trevally and Ocean Jackets for assessment and management

John Stewart, Ash Fowler, Rowan Chick, Amy Smoothey, Julian Hughes, Charlie Huveneers, Patrick Reis-Santos, Bronwyn M. Gillanders, Rhiannon Van Eck, Corey Green, Sean Tracey, Nils Krueck, Michael Drew, David V. Fairclough, Dan Corrie, Jonathan Sandoval-Castillo, Zoriana H. T. Lam, Luciana M. Möller and Luciano B. Beheregaray

December 2024

FRDC Project No **2021-009**

ISBN: 978-1-76058-854-0

Identifying biological stocks of Silver Trevally and Ocean Jackets for assessment and management.

FRDC Project No 2021-009

Year of Publication: 2024

Researcher Contact Details
Name: Dr John Stewart
Address: New South Wales Department of Primary Industries and Regional Development
Sydney Institute of Marine Science
19 Chowder Bay Road
Mosman, NSW, 2088
Phone: 0438 249 147
Email: John.Stewart@dpi.nsw.gov.au

FRDC Contact Details
Address: 25 Geils Court
Deakin ACT 2600
Phone: 02 6122 2100
Email: frdc@frdc.com.au
Web: www.frdc.com.au

Contents

Tables

Figures

Acknowledgments

We thank the following staff from the collaborating organisations for their contributions to this project:

Flinders University. We thank Diana Elena-Vornicu for support with laboratory and data processing. We thank Dr Maren Wellenreuther for provision of Silver Trevally samples from New Zealand.

The University of Adelaide. We thank Dr Sarah Gilbert and acknowledge the instruments and expertise of Microscopy Australia at Adelaide Microscopy, The University of Adelaide, enabled by NCRIS, University, and State government support.

New South Wales Department of Primary Industries and Regional Development. We thank Chantelle Clain and Nick Meadows for support with sample collection, otolith processing, and co-ordination between collaborators.

We acknowledge and thank the contribution of commercial and recreational fishers who assisted in provision of samples for the project. In particular, we thank Danny Stewart, Mark Cranstone, Scott Westly, Rocky Pirrello, Ben McCulloch, and John Jarvis.

Victorian Fisheries Authority. We thank Mr Scott Gray for providing samples from western Victoria.

Department of Primary Industries and Regional Development (DPIRD) WA. We thank numerous staff for sample collection, particularly Elaine Lek.

Executive Summary

This project examined the biological stock structure of Silver Trevally (*Pseudocaranx georgianus*) and Ocean Jackets (*Nelusetta ayraud*) in Australia. The assessment of stock structure was facilitated through a contemporary sampling regime co-ordinated through the New South Wales Department of Primary Industries and Regional Development (NSW DPIRD). The project was a national collaboration between the FRDC and all relevant jurisdictions including NSW DPIRD, Victorian Fisheries Authority (VFA), University of Tasmania (UTAS), South Australian Research and Development Institute (SARDI), Western Australian Department of Primary Industries and Regional Development (DPIRD), Australian Fisheries Management Authority (AFMA), The University of Adelaide and Flinders University. The project results will be important for future cross-jurisdictional monitoring, assessment, and management of these high-priority fish stocks.

Background

Silver Trevally and Ocean Jackets support important commercial and recreational fisheries across the southern half of Australia. Despite their abundance and long history of being fished, relatively little is known about their stock structures or population demographics. As a result, assessments of these species have historically been done at local scales to service local management needs.

The national Status of Australian Fish Stocks (SAFS) initiative, supported by the FRDC and the Australian Fisheries Management Forum (AFMF) since 2012, aims to assess the status of Australia's key wild catch fish stocks at the 'biological stock' level. In doing so, SAFS promotes cross-jurisdictional collaboration to report on the status of fish stocks at a meaningful biological scale rather than at the spatial scale of the management agency. Several species, including Silver Trevally and Ocean Jackets, have been identified as important species that have historically been assessed at the jurisdictional or management unit scale through SAFS, but may require future assessments to be done at the preferred and more meaningful level of the biological stock. The working definition of a biological stock is "a genetically or functionally discrete population that is largely distinct from other populations of the same species and can be regarded as a separate homogeneous group for management or assessment purposes". There is therefore a clear need for the biological stock structures of Silver Trevally and Ocean Jackets to be defined nationally to support future assessments.

Aims/objectives

The project aimed to improve future stock status assessments for Silver Trevally and Ocean Jackets, and future iterations of SAFS, through achieving the following objectives:

1. To clarify the stock structure of Silver Trevally nationally across Queensland, New South Wales, Victoria, Tasmania, South Australia, Western Australia, and the Commonwealth.

2. To clarify the stock structure of Ocean Jackets nationally across New South Wales, Victoria, Tasmania, South Australia, and the Commonwealth.

3. Using information from objectives 1 and 2, make recommendations on stock delineation and the appropriate scales for management for each species.

Methodology

The project assessed information available for both species and generated new data through biological sampling across their distributions. This sampling encompassed all relevant jurisdictions and major fishing ports.

Otolith-based assessments

A considerable number of otoliths were sampled from across the distributions of Silver Trevally (n = 940) and Ocean Jackets (n = 451). Otolith characteristics in terms of shape and chemistry were analysed for variations between sampling locations and used to infer connectivity during lifetime temporal scales. Otolith shape was described using discrete Wavelet analysis of contours based on outlines of otoliths. Otolith chemistry was assessed in terms of trace elements using laser ablation inductively coupled plasma mass spectrometry (LA ICP-MS). Datasets were analysed using multivariate techniques.

Genomic assessments

Tissue samples of Silver Trevally (n = 929) and Ocean Jackets (n = 465) were taken from fish across their distributions. DNA was extracted and sequenced to produce thousands of genome-wide, highly resolving DNA markers known as single-nucleotide polymorphisms (SNPs). Genomic diversity was assessed using the number of polymorphic loci and estimation of observed (Ho) and expected (He) heterozygosity by locality for each species. Stock structure for Silver Trevally and Ocean Jackets was assessed based on Principal Component Analysis (PCA) and Admixture analyses.

Results/key findings

Using a combination of genomic and otolith-based approaches, the study revealed strong evidence for the existence of two similarly distributed biological stocks for Silver Trevally and Ocean Jackets.

Silver Trevally comprise two distinct biological stocks in Australia. The 'western' biological stock extends from Western Australia to western Victoria, whereas the 'eastern' biological stock extends from eastern Victoria to northern New South Wales, including north-eastern Tasmania and Commonwealth-managed waters off New South Wales. The western and eastern biological stocks are likely separated by Bass Strait.

Ocean Jackets also comprise two distinct biological stocks in Australia. The 'western' biological stock extends from Western Australia to western Victoria, whereas the 'eastern' biological stock extends from northern New South Wales south to eastern Victoria, Tasmania, and Commonwealth managed waters east of these states. These stocks are also likely separated by Bass Strait.

Silver Trevally and Ocean Jackets both exhibited variations in otolith characteristics that resulted in high classification rates to some locations, suggesting potential for finer-scale population structuring and/or habitat use within the broader biological stocks identified. Future research will be required into the population dynamics and life-history parameters of both species to assess whether finer-scale population structuring is important for assessment and management.

Implications for relevant stakeholders

The findings that western and eastern biological stocks of Silver Trevally and Ocean Jackets span multiple jurisdictional boundaries will increase the need for cross-jurisdictional collaboration in monitoring, assessment, and management, of these spatially extensive stocks.

Recommendations

Future iterations of SAFS should assess Silver Trevally by biological stock, being a western biological stock (fished in Western Australian, South Australian, western Victorian waters) and an eastern biological stock (fished in New South Wales, east-coast Commonwealth, eastern Victorian and Tasmanian waters).

Similarly, future iterations of SAFS should assess Ocean Jackets by biological stock, being a western biological stock (fished in Western Australian, South Australian, Commonwealth Great Australian Bight Trawl, and western Victorian waters) and an eastern biological stock (fished in New South Wales, east-coast Commonwealth and eastern Victorian waters).

Future biological stock status assessments should be done through cross-jurisdictional collaboration in terms of monitoring, data collection, and stock assessment for Silver Trevally and Ocean Jackets.

Keywords

Silver Trevally, *Pseudocaranx georgianus*, Ocean Jackets, *Nelusetta ayraud*, stock structure, otolith shape, otolith chemistry, genomics.

Introduction

This project was developed in response to an FRDC call for research into 'Resolving stock uncertainty for priority species' in 2021. Five priority species were listed by the FRDC: Mangrove Jack (*Lutjanus argentimaculatus*), Silver Trevally (*Pseudocaranx georgianus*), Giant Spider Crab (*Leptomithrax gaimardii*), Ocean Jackets (*Nelusetta ayraud*), and Giant Crab (*Pseudocarcinus gigas*). These five species were considered priorities for stock structure research to improve the national Status of Australian Fish Stocks (SAFS) initiative that has been ongoing since 2012. These five species have been assessed at either the jurisdictional or management unit level due to insufficient specific knowledge on the spatial scale of biological stock structure. It is best practice to assess stocks at the biological stock level and it is a goal of the FRDC and the Australian Fisheries Management Forum (AFMF) to continually improve SAFS and the national assessment of fish stocks.

Of the five priority species listed, Silver Trevally and Ocean Jackets are important finfish to commercial and recreational fishers across the southern half of Australia. Consequently, all fisheries management agencies across temperate Australia supported development of this project to specifically clarify the stock structure of Silver Trevally and Ocean Jackets in this region.

In addition to supporting future iterations of SAFS, improved knowledge of the stock structure of these two species will assist monitoring, assessment, and management across their distributions. Silver Trevally in eastern Australia were assessed in SAFS 2020 as being 'Depleted' in New South Wales, and 'Sustainable' in Commonwealth, Victorian, and Tasmanian waters. If, however, there is a single east-coast biological stock of Silver Trevally there will need to be improved cross-jurisdictional assessment and management to recover the stock. Ocean Jackets have been identified as an emerging species for commercial fisheries in southern Australia and were assessed in SAFS 2020 as 'Sustainable' in New South Wales, South Australia, Southeast Scalefish and Shark Fishery, and Great Australian Bight Trawl Sector, 'Negligible' in Tasmania, and 'Undefined' in Victoria. Ocean Jacket is the focus of current FRDC-funded work supporting stock assessments of emerging species (FRDC 2022-032).

Objectives

1. To clarify the stock structure of Silver Trevallies nationally across Queensland, New South Wales, Victoria, Tasmania, South Australia, Western Australia, and the Commonwealth.

2. To clarify the stock structure of Ocean Jackets nationally across New South Wales, Victoria, Tasmania, South Australia, and the Commonwealth.

3. Using information from objectives 1 and 2, make recommendations on stock delineation for each species and the appropriate scales for management.

Method

This study used otolith and genomic based techniques to understand the stock structure of Silver Trevally and Ocean Jackets nationally. The sampling design was agreed upon by the project team, recommending at least 30 fish from any one location would be sufficient for both the otolith and genomic work. The initial target was to sample 40 fish per species from each location to allow for unusable samples, with otoliths and tissue being sampled from the same fish where possible. Locations were selected based on the distribution of the fisheries nationally, where major fishing ports were located, and to test specific hypotheses around stocks of fish being harvested by state and Commonwealth fisheries operating from the same ports. The aim was to sample fish during a three-month window, October to December 2021 for Ocean Jackets, and January to March 2022 for Silver Trevally (Figs. 1 and 2). These time periods were chosen based on patterns in commercial landings and optimising the likelihood of being able to gain samples at similar times across the entire distribution of each species.

Sampling was co-ordinated by the NSW DPIRD, with collaborators in each jurisdiction being responsible for collection of samples from their respective region. All fish were measured to the nearest mm. Ocean Jackets were measured as total length (TL) and Silver Trevally as both fork length (FL) and TL. In cases where only one length measurement was made for Silver Trevally, the associated FL or TL was estimated using the linear FL to TL relationship derived from unpublished monitoring data in NSW:

$FL = 0.816 \times TL + 0.870$, $r^2 = 0.995$

Fish were weighed to the nearest gram (g) and when possible, sex identified by the presence of ovaries or testes. Otoliths were extracted, cleaned, and stored dry in envelopes. Tissue (muscle) samples were initially taken using a purpose-made tissue sampling unit comprising a punch gun and vials containing buffered preservative (see Allflex Tissue Sampling - 4Tags.com.au). This approach attempted to standardise sampling across all jurisdictions.

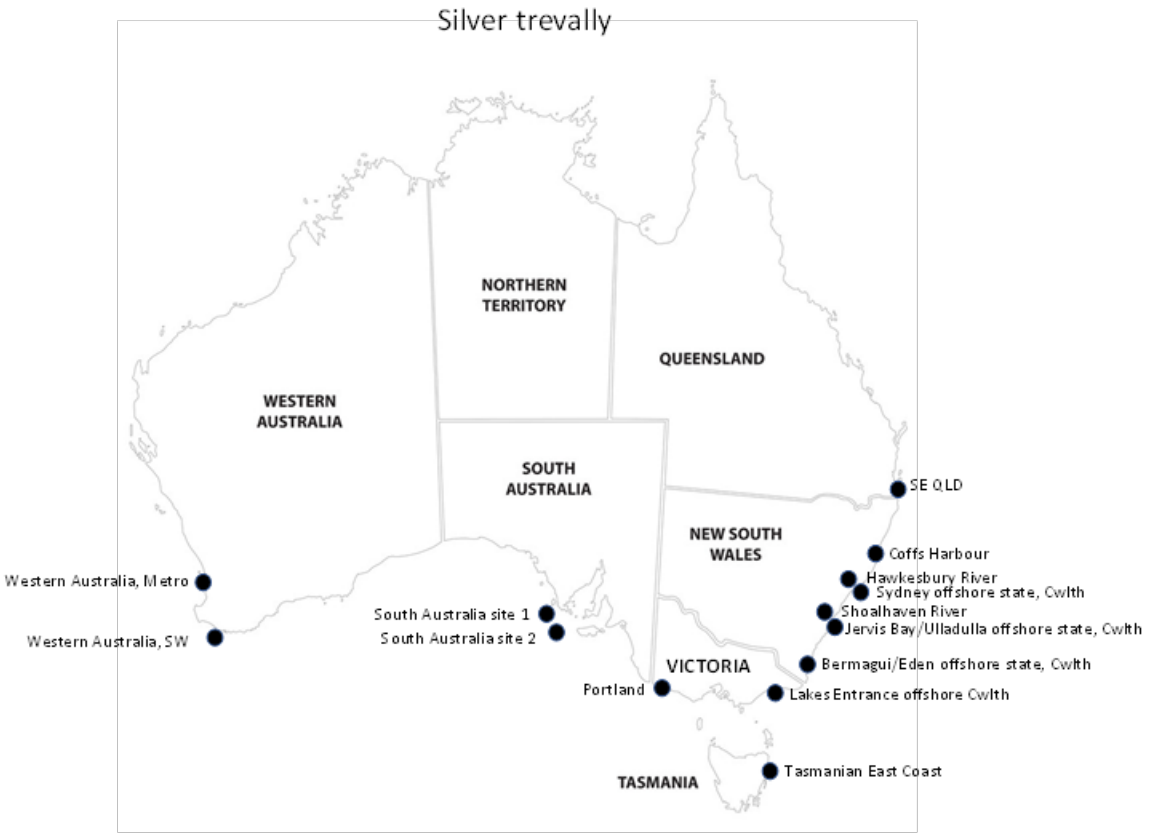

Figure 1. Initial sampling design for Silver Trevally. The target was for 40 fish per location.

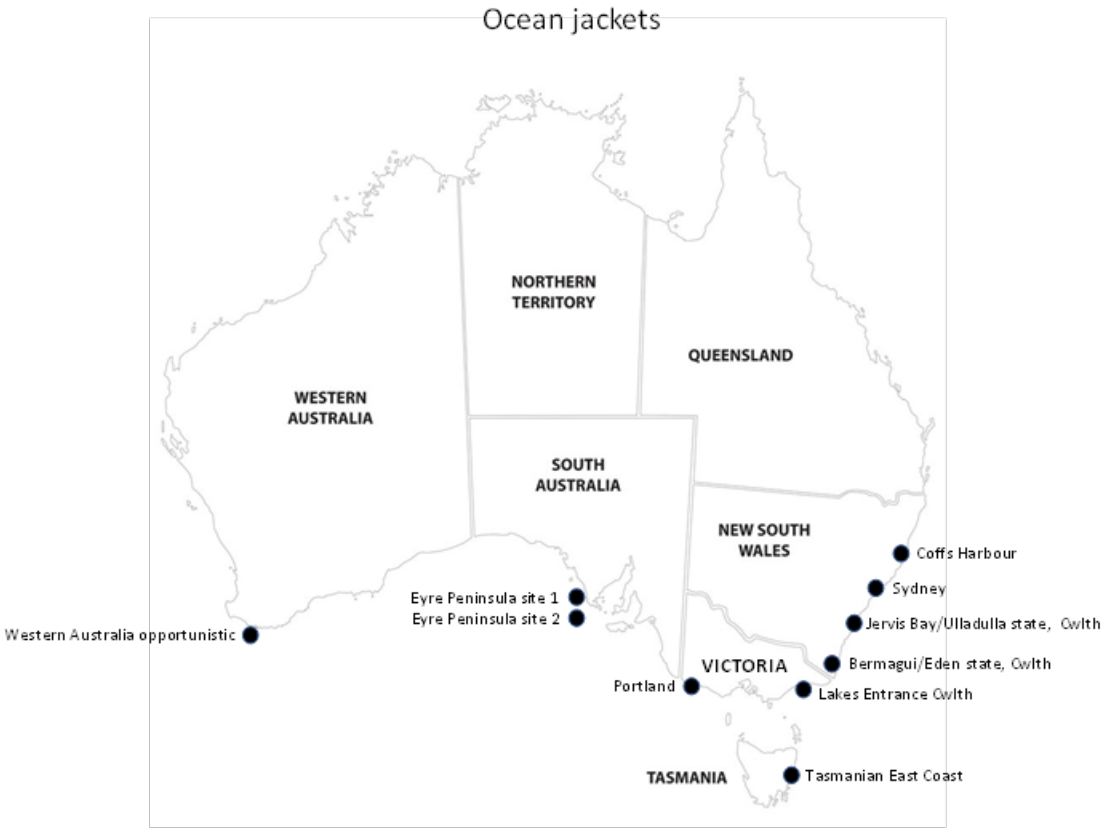

Figure 2. Initial sampling design for Ocean Jackets. The target was for 40 fish per location.

Pilot analyses found very poor-quality genetic material in many of the samples taken using the tissue sampling units, to the extent that it was not as useful as required. Many of these samples had been taken from fresh fish and the tissue handled and stored according to best practice. It was assumed that the buffer solution in some vials was of poor quality and did not preserve the tissue adequately. Subsequently, a second round of tissue sampling was done to get sufficient samples of high-quality genetic material. This second round of sampling was done during the remainder of 2022, with tissue being taken using clean scalpel blades and then being stored in 100% ethanol.

Otolith-based methods

The project team collected otoliths from 940 Silver Trevally and 451 Ocean Jackets for analyses (Tables 1 and 2). Here, we assessed patterns of variation in otolith shape and chemical composition, and if they discriminated among population units collected along the species' geographical distribution gradient, to obtain information on the underlying population structure.

Table 1. Summary of otoliths sampled from Silver Trevally by jurisdiction and location. FL = fork length.

Jurisdiction	Location	No. of Silver Trevally	Size range (mm FL)
Commonwealth	Bermagui	71	161 — 326
	Sydney	78	175 — 276
	Ulladulla	69	180 — 290
New South Wales	Ballina	2	238 — 252
	Bermagui	23	251 — 310
	Coffs Harbour	40	255 — 304
	Jervis Bay	40	277 — 480
	Shoalhaven Coast	22	259 — 407
	Shoalhaven River	20	249 — 345
	Sydney	40	249 — 292
Victoria	Corner Inlet	61	175 — 276
	Port Fairy	52	210 — 400
Tasmania	Low Head	29	207 — 368
	North Tasmania	40	222 — 288
	St Helens	10	297 — 462
South Australia	Cape Elizabeth	14	310 — 405
	North Neptune	30	306 — 454
	South Neptune	11	262 — 376
	Venus Bay	32	296 — 475
Western Australia	Metropolitan	60	213 — 404
	Mid-west	21	231 — 366
	South Coast (East)	63	164 — 588
	South Coast (West)	60	172 — 335
	South-west	52	178 — 368
Total		**940**	**161 — 588**

Table 2. Summary of otoliths sampled from Ocean Jackets by jurisdiction and location. TL = total length.

Jurisdiction	Location	No. of Ocean Jacket	Size range (mm TL)
Commonwealth	Eden	40	196 — 313
	Greencape	20	304 — 361
	Ulladulla	38	291 — 376
New South Wales	Coffs Harbour	40	272 — 451
	Jervis Bay	40	275 — 372
	Narooma	40	312 — 380
	Terrigal	39	295 — 415
Victoria	Portland	42	326 — 420
South Australia	Coffin Bay	46	292 — 565
	Port Lincoln	37	326 — 420
Western Australia	Metropolitan	1	681
	South-west	68	554 — 734
Total		**451**	**196 — 734**

Otolith shape

Otoliths were inspected and cleaned prior to imaging, ensuring their outline was intact and no foreign material was present. Only whole undamaged otoliths were selected (i.e., no damage or vateritic material), as this can alter otolith morphometric analyses, and where possible the left otolith was prioritised, aiming for thirty selected otoliths per source location. Otoliths were imaged whole using reflected light against a black background with a dissection microscope (Olympus SZ61) and image analysis system (Teledyne QImaging Micropublisher^{tm} 5.0 RTV). Otoliths were positioned with the proximal surface facing upwards and the sulcul axis aligned horizontally during imaging (Fig. 3). For both species, when left otoliths were damaged right otoliths were selected where necessary to ensure enough samples were obtained. Images for right otoliths were mirrored, with preliminary tests indicating no differences between right and left otoliths. All images were converted to monochrome and edited to enhance contrast and maximise otolith outlines in Adobe Lightroom (Fig. 4).

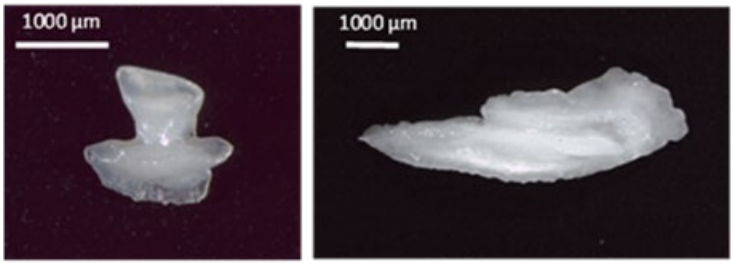

Figure 3. Otoliths of Ocean Jacket (left) and Silver Trevally (right) from Coffs Harbour, NSW.

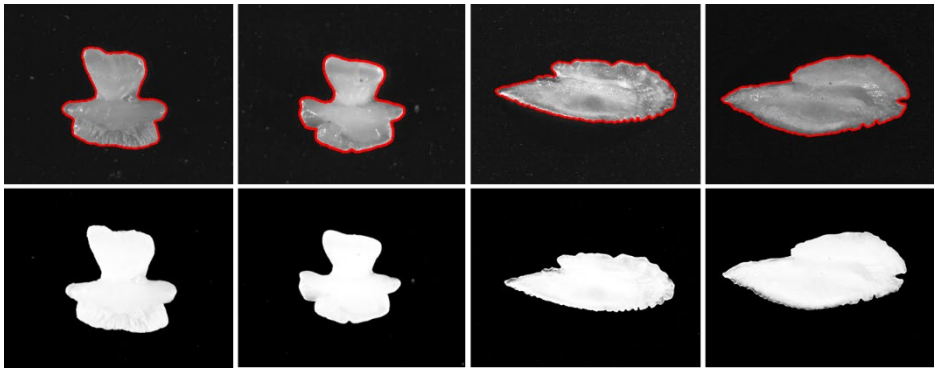

Figure 4. Examples of otolith outline tracing (top) generated in ShapeR and of monochrome and high contrast edited images (bottom). From left to right, Ocean Jacket otoliths from Coffs Harbour NSW and Coffin Bay SA, and Silver Trevally otoliths from Albany WA and Sydney Commonwealth.

The ShapeR package (Libungan and Palsson, 2022) was used in R version 4.1.1 (R Core Team, 2023) to process all otolith images and obtain shape outlines along with multiple morphometric parameters (i.e., perimeter, area, length, and width). Contour smoothing of the outlines was performed to reduce the risk of interaction from pixel noise in the analysis (Libungan and Palsson, 2015). Outlines were transformed into shape coefficients using discrete wavelet analysis, a powerful and best-suited approach for approximating sharp edges (Libungan and Palsson, 2015). Each shape coefficient was adjusted for allometric relationships with fish length, and the quality of wavelet reconstruction was estimated by comparing the deviation from the otolith outline (i.e., if each outline matched the otolith shape). Where necessary, image enhancement was used to allow for the inclusion of the largest number of samples possible, e.g., if there were deviations from the otolith outline due to issues of transparency of the otolith material, the images were further manipulated to improve the contrast between the otolith and background. The average otolith outline per source group was plotted, along with variations in wavelet coefficients along polar coordinates to evaluate the differences in shape both within and among source group's locations (Limbungan and Palsson, 2015).

To reduce the number of coefficients required to sufficiently reconstruct the otolith outline and to allow integrating analysis of otolith shape and chemistry, otolith shape coefficient data were transformed into principal component (PC) scores where the minimum number of required scores was selected based on scree plots and a cut off threshold of 70% cumulative total variation (Jolliffe, 2002). The Principal Component Analysis (PCA) and scree plots were performed using the factoextra (Kassambara and Mundt, 2020), ggplot2 (Wickham, 2016), and readxl (Wickham and Bryan, 2019) packages in R (R Core team, 2023).

Given otolith shape reflects environmentally driven phenotypic traits, groups of fish with different outlines of their otoliths (otolith shape) can be considered separate management units (Izzo et al., 2017, Stransky, 2014). We compared otolith shape across locations but also among regions and states (pooling locations) to investigate broad-scale patterns and test hypotheses of differences in structure at different spatial scales. To infer stock structure across source locations, permutational multivariate analysis of variance (PERMANOVA) and canonical analysis of principal coordinates (CAP) were used to compare locations and evaluate classification success (see data analysis for more details).

Otolith chemistry

Where possible the left otolith was used for chemical analysis following imaging for shape analysis. Thirty otoliths from each location were embedded in blocks of Indium spiked epoxy resin in rows of five and sectioned transversely through their primordium, using a Gemmasta^{tm} lapidary saw fitted with a lubricated diamond edged blade. Strips of epoxy containing the otolith sections were ground and polished using 9 μm and 3 μm lapping film, before being glued to standard slides using thermoplastic cement. Approximately 100 otolith sections were glued onto each slide.

All slides were sonicated in ultrapure water for three minutes, triple rinsed with ultrapure water, and dried in a laminar flow before analysis. Chemical analyses were performed via Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA ICP-MS) and followed procedures in Reis-Santos et al. (2018) and Jackson et al. (2024). Elemental concentrations of 11 elements (^{7}Li, ^{11}B, ^{23}Na, ^{24}Mg, ^{55}Mn, ^{63}Cu, ^{66}Zn, ^{88}Sr, ^{138}Ba, ^{208}Pb) were analysed using a RESOlution LR 193nm Excimer laser system attached to an Agilent 7900x inductively coupled plasma mass spectrometer (LA ICP-MS). ^{43}Ca was measured as an internal standard to correct for variation in ablation yield. ^{115}In was measured as a marker to identify potential contamination from epoxy resin and crystalbond. Thirty μm spots were used to analyse the marginal edge of the otoliths, i.e., recent elemental incorporation representative of time and location of capture (approx. the last six and five months for Silver Trevally and Ocean Jackets, respectively, though there is variation with fish size and age). Laser ablations (5hz, fluence ~3.5 j cm^{2}) occurred in a sealed chamber with the resulting analyte transported to the ICP-MS via a smoothing manifold in an argon (Ar) and helium (He) stream. Pre-ablations were done to remove any potential surface contamination, followed by 30 s of background reading and 40 s of laser ablation.

Certified reference material NIST 612 (National Institute of Standards and Technology) was analysed at the start and end of each session and repeatedly throughout the laser sessions to correct for mass bias and machine drift. Another calcium carbonate certified standard material, MACS-3 (United States Geological Survey), was run at the beginning and end of each session to check long-term consistency and external precision of elemental readings. Mean recovery for NIST 612 was 100 % for all elements (between 99.99 % and 100.07 %). Precision and coefficient of variation (% relative standard deviation, RSD) ranged between 0.3 % and 1.0 % for NIST612, and 2.8 % to 10.0 % for MACS-3.

All acquired raw data was reduced, including background corrections and mass count to ppm conversions using Iolite (Paton et al., 2011), with samples evidencing contamination removed from subsequent data analysis. Data in ppm were then converted to Element:Ca ratios (μmol/mol) using the atomic mass of the element and the % Ca content of the calcium carbonate matrix concentration of otoliths (Yoshinaga et al., 2000).

Similar to otolith shape analysis, PERMANOVA was used to assess differences in chemical composition among locations and regions of capture. Classification statistics were generated using CAP, to evaluate the classification success in determining stock structure based on otolith chemical composition across source

sites and regions (i.e., pooling adjacent collection locations within regions) (see data analysis for more details).

Data analysis

We compared otolith shape coefficients and Element:Ca data across locations but also among regions and jurisdictions (pooling collection locations) to investigate fine- and broad-scale patterns and test hypotheses of differences in structure at different spatial scales for each species.

Data were normalised and transformed to Euclidean distance resemblance matrices, and permutational multivariate analysis of variance (PERMANOVA, Primer v7) (Anderson, 2001) was used to assess differences in otolith shape and chemistry among locations and regions (by pooling collection locations). When significant differences among regions were identified, pairwise analyses were undertaken to assess differences among adjacent or regional populations. Canonical analysis of principal coordinates (CAP) was used to investigate otolith shape and chemical variation among locations and regions (by pooling collection locations) for each species in Primer v7 (Libungan and Palson, 2015, Anderson and Willis, 2003). Classification of individuals to locations and regions were generated using the 'leave one-out' approach (Anderson and Willis, 2003, Izzo et al., 2017, Jackson et al., 2023). This is a constrained ordination for discriminating among a priori groups and provides a sound and unbiased measure of how distinct groups are in multivariate space. Overall, CAP was used to evaluate habitat use of individual fish and the classification success in discriminating adjacent population units along the species' geographical distribution gradients. Analyses were applied to individual locations as well as by pooling fish from different geographical locations, with groupings ranging from adjacent locations to increasingly larger regional scales (e.g., among and within jurisdictions or sections of the coast). We also ran an integrated analysis with otolith shape and chemical data combined, with the combined data prepared and run in the same manner. Chemical data were transformed into PC scores to allow integration with the otolith shape PC scores. This was undertaken to examine if the combination of both morphometry and chemistry would strengthen the analysis and allocation of samples per location.

Genomics methods

A short instructional video was produced showing how to use the punch gun and sample vials, as well as where to take the tissue sample from. This was distributed to all jurisdictions. The preferred areas to take tissue from Silver Trevally and Ocean Jackets are shown in Figs. 5 and 6. Samples, whether preserved in vials with 100% ethanol or in Allflex® Tissue Sampling Unit (TSU) units, were shipped to the Molecular Ecology Lab., Flinders University (MELFU) for genomic analyses.

Figure 5. Location of tissue sampling using the punch guns for Silver Trevally.

Figure 6. Location (blue dot) of tissue sampling using the punch guns for Ocean Jackets.

The project team collected tissue samples from 929 Silver Trevally and 465 Ocean Jackets for analyses (Tables 3 and 4).

Table 3. Summary of tissue sampled from Silver Trevally by jurisdiction and location. FL = fork length.

Jurisdiction	Location	No. of Silver Trevally	Size range (mm FL)
Commonwealth	Bermagui	71	196 — 370
	Ulladulla	69	202 — 335
	Sydney	80	208 — 311
New South Wales	Bermagui	23	302 — 375
	Jervis Bay	40	322 — 528
	Shoalhaven Coast	22	299 — 476
	Shoalhaven River	20	298 — 408
	Sydney	40	293 — 340
	Coffs Harbour	40	306 — 363
	Ballina	4	260 — 305
Victoria	Corner Inlet	71	182 — 293
	Port Fairy	41	180 — 340
Tasmania	Low Head	29	246 — 436
	North Tasmania	40	260 — 341
	St Helens	10	356 — 547
South Australia	Cape Elizabeth	14	240 — 320
	Cape Jervis	2	457 — 476
	North Neptune	30	375 — 555
	Venus Bay	32	242 — 377
Western Australia	Metropolitan	61	250 — 495
	Mid-west	21	283 — 448
	South Coast (East)	62	201 — 720
	South Coast (West)	55	210 — 410
	South-west	52	218 — 450
Total		**929**	**180 — 720**

Table 4. Summary of tissue sampled from Ocean Jackets by jurisdiction and location. TL = total length.

Jurisdiction	Location	No. of Ocean Jacket	Size range (mm TL)
Commonwealth	Eden	40	196 — 376
	Greencape	20	304 — 361
	Ulladulla	60	291 — 376
New South Wales	Narooma	40	312 — 380
	Jervis Bay	40	275 — 372
	Terrigal	39	295 — 416
	Coffs Harbour	40	272 — 451
	Ballina	1	220
Victoria	Portland	30	315 — 480
South Australia	Coffin Bay	46	292 — 565
	Port Lincoln	38	326 — 420
Western Australia	Metropolitan	1	681
	South-west	70	554 — 734
Total		**465**	**196 — 734**

DNA extractions and genomic sequencing

Genomic DNA of representative Silver Trevally (n=90) and Ocean Jackets (n=54) were initially extracted at MELFU to assess sample quality, using a modified salting-out method (Sunnucks and Hales, 1996). Test runs of both species indicated variable quality and yield of DNA as measured by a NanoDrop 1000 spectrometer (Thermo Scientific) and a Qubit fluorometer, respectively. Silver Trevally and Ocean Jacket samples with high DNA yield and others with a clear preserving agent were prepared into seven and four (96-well) plates, respectively, for DNA extraction and sequencing via DArTseq® (Diversity Arrays Technology Pty Ltd) (Jaccoud et al., 2001). This methodology is a genome complexity reduction-based technology that produces thousands of genome-wide, highly resolving DNA markers known as single-nucleotide polymorphisms (SNPs). Briefly, it applies a combination of methylation-sensitive restriction enzymes, compatible adaptors and attachment sequences for ligation within an Illumina flow cell (Jaccoud et al., 2001).

Data analyses

SNP filtering

Raw sequence reads and standardly filtered SNP datasets for each fish species were received from DArT after sequencing. Quality checks of the datasets and additional filtering steps using the MELFU standard bioinformatics were implemented to retain only high-quality loci and samples for analyses of stock structure of the two fish species. These include criteria to exclude loci that were monomorphic; had a large amount of missing data (>20%); where the minor allele frequency was <1% and that exhibited <99% reproducibility; were called in <80% of individuals; had a depth of coverage <5; exhibited significant departures from Hardy-Weinberg disequilibrium in >2 locations. In addition, only the best SNP of each sequencing fragment was used for analyses (see Tables 9 and 14 in relevant species chapters).

Genomic diversity

Analyses of genomic diversity included an assessment of the number of polymorphic loci and estimation of observed (Ho) and expected (He) heterozygosity by locality for each fish species, using the dartR package in R (version 4.3.1) (Gruber et al., 2018).

Stock structure

Stock structure for the two species of interest was assessed based on two methods.

Principal Component Analysis (PCA) is a model-free approach for investigating population structure, which is readily available within the package Adegenet (Jombart and Ahmed, 2011). PCA is a multivariate analysis based on overall variance, which reduces the dimensionality of the dataset while maximising genomic similarity (Abdi and Williams, 2010). The analysis infers principal components (PCs) carrying a certain amount of variance, with the PCs obtained through the calculation of eigenvalues, and the percentage of variances of each PC also calculated to determine the number of dimensions involved. After analysis in Adegenet, the PCA plot was created using the R ggplot2 package by plotting the PCs carrying the highest significance of variances (Novembre and Stephens, 2008).

Admixture is a maximum likelihood method to infer population stratification based on estimated individual ancestries and on a fast sequential quadratic algorithm (Alexander et al., 2009). After initial Admixture analysis, a cross-validation procedure was employed to identify the most likely value for the number of populations (K). Five-fold cross-validations were done for K values (1-[#locations + 1]), and the most likely K was chosen based on the lowest associated cross-validation (CV) error. A graphic output of the ADMIXTURE results was then compiled in ggplot2.

Both PCA and Admixture analyses do not use a priori information about location or region, and therefore are ideal to disclose sub-structure, if present, at any geographical scale.

Genetic differentiation

Classic fixation indices, which are related to the variance in allele frequencies, were also measured to obtain information about levels of genetic differentiation between localities. These were estimated between pairs of localities using the dartR package (95% confidence intervals determined based on 9,999 bootstraps; p-values adjusted considering a 5% False Discovery Rate (FDR, q-value) (Benjamini and Yekutieli, 2001). The resulting FST values were then visualised by a heatmap generated by the ggplot2 package (Wickham, 2016).

Isolation by distance

Isolation-by-distance (IBD) was investigated by testing the relationship between genetic (Fst/(1- Fst)) and geographical distances across sampling localities using the Mantel test available within the dartR package, with 10,000 permutations (Gruber et al., 2018; Mantel, 1967; Mijangos et al., 2022). For the tests, the shortest geographical distances between sampling localities following the coastline were calculated using the viamaris function in melfuR version 0.9 (https://github.com/pygmyperch/melfuR). Synthetic coordinates created using the isoMDS function in the MASS package (Venables and Ripley, 2002) were obtained to represent coordinates for calculating distance matrices when performing the tests. Tests were performed at a continental level, and separately for each population identified by the PCA and Admixture analyses.

Stock structure of Silver Trevally *Pseudocaranx georgianus*

Introduction

Prior to 2006, Silver Trevally in Australia were classified as *Pseudocaranx dentex,* except for Sand Trevally *Pseudocaranx wrighti* that is morphologically distinct (Bearham et al., 2020). However, recent morphological work has demonstrated this to be a species complex, comprising three distinct species*: Pseudocaranx georgianus*, *Pseudocaranx dinjerra* and provisionally *Pseudocaranx sp. dentex* recorded in Queensland and Lord Howe Island (Smith-Vaniz and Jelks, 2006). *Pseudocaranx georgianus* (hereafter Silver Trevally) is distributed from northern New South Wales to Western Australia, south of 25˚S latitude (Bearham et al., 2020). *Pseudocaranx georgianus* also occurs in New Zealand where it supports substantially larger fisheries than those in Australia, with a commercial allocation in the order of 4,000 t p.a. (Kemp, 2020); whereas commercial landings in Australia peaked at around 1,600 t in 1990 and have since declined to less than 100 t p.a. (Burch et al., 2023).

Silver Trevally inhabit coastal and estuarine environments, and are important components of recreational, indigenous, and commercial fisheries nationally. The stock structure of Silver Trevally is poorly understood and has resulted in the species being assessed separately by individual jurisdictions (Fowler et al., 2021). Justification for assessing Silver Trevally at the jurisdictional level has included evidence of limited movements of adults from tag-recapture studies in New Zealand, New South Wales, and Western Australia (James, 1980, Fairclough et al., 2011, Fowler et al., 2018). Nevertheless, without an understanding of biological stocks and their distributions the decision to assess by individual jurisdiction may be high risk in terms of sustainable management. In fact, an inability to reconcile contradictory stock status assessments by different jurisdictions off eastern Australia due to uncertainty around stock structure was one major driver for initiating this project.

Several life-history traits documented off eastern Australia suggest potential for substantial dispersal of eggs and larvae. Silver Trevally is a schooling species, with an extended spawning season from September to March and spawning occurring throughout the range of the population (Rowling and Raines, 2000). Silver Trevally mature at relatively small sizes (around 19 cm FL) and have high fecundity (Rowling and Raines, 2000). They are partial spawners, releasing several batches of pelagic eggs over a spawning season (Annala et al., 1999, Neira and Miskiewicz, 1998, Farmer et al., 2005). Given the dominance of the southerly flowing Eastern Australian Current off eastern Australia during the spawning season, it is highly likely that eggs and larvae are widely dispersed, hence promoting the notion of genetic homogeneity in this region. Similarly in Western Australia Silver Trevally spawn through approximately a five-month period from August to December (Farmer et al., 2005). The size at sexual maturity has been reported to be slightly larger in Western Australia at approximately 310 mm and 279 mm TL for females and males respectively (Farmer et al., 2005).

Otolith-based assessment

Results

Otolith shape

Wavelet shape reconstructions of whole Silver Trevally otoliths generated 63 shape coefficients and revealed average morphometric variations among locations and jurisdictions (Fig. 7).

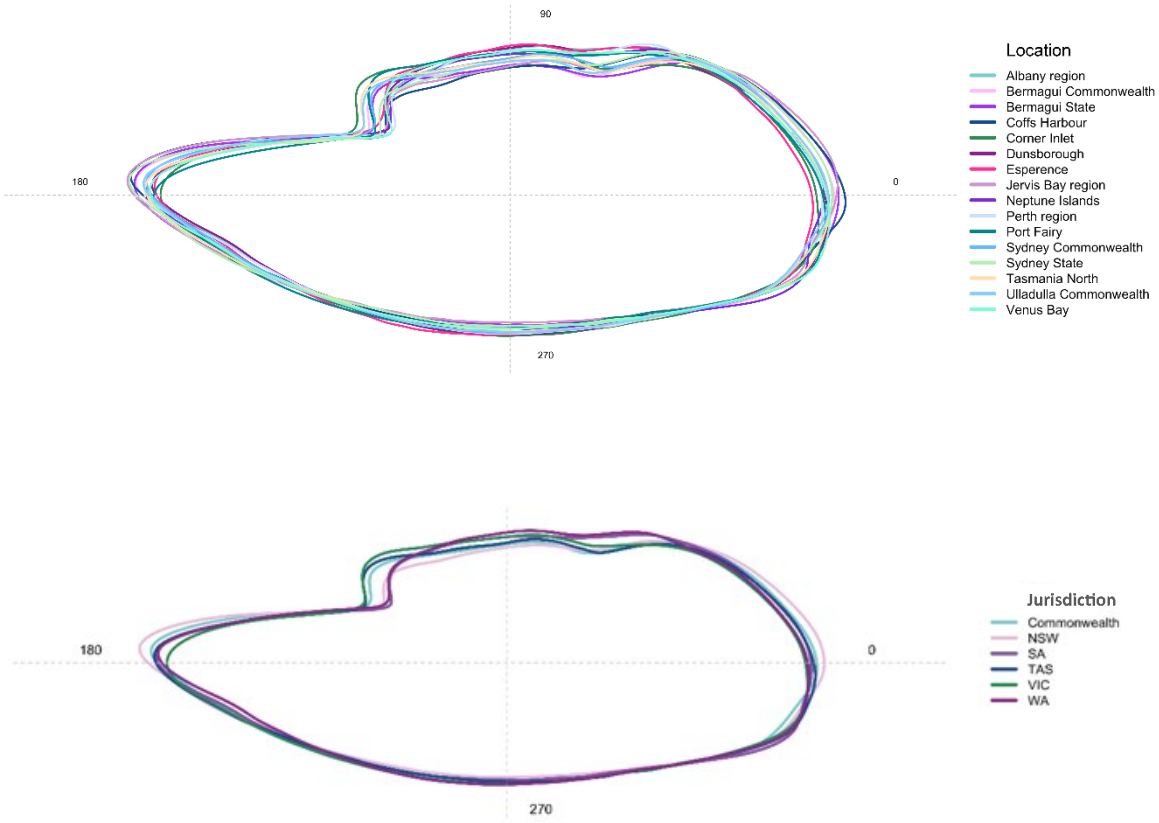

Figure 7. Discrete wavelet analysis of Silver Trevally showing (top) mean otolith shape for each location, and (bottom) mean otolith shape for each jurisdiction.

Variability and differences among locations and jurisdictions were noticeable in the rostrum, excisura, antirostrum and dorsal lobe. Overall, we see significant differences among locations (Pseudo-$F_{(15, 567)}$ = 3.1, P<0.001), but overall classification across the entire collection range was low (21 %), with the majority of locations having classification accuracies <20 %, and classification accuracies maximum at 60 and 40 % accuracies for Corner Inlet and Port Fairy, in eastern and western VIC, respectively (Table 5). While PERMANOVA is a powerful tool to compare group means, it does not assess or reflect the ability to classify samples to groups of origin. Low classification accuracies are likely due to complex interactions that result in overlapping data in multivariate space, limiting distribution in multivariate space and the identification of patterns for accurate group predictions. Nonetheless, CAP analysis and sample distribution across the multivariate space highlight trends among jurisdictions and broader east to west regions that were further explored in subsequent analysis by pooling locations by jurisdictions and broader regions (Figs. 8, 9 and appendices).

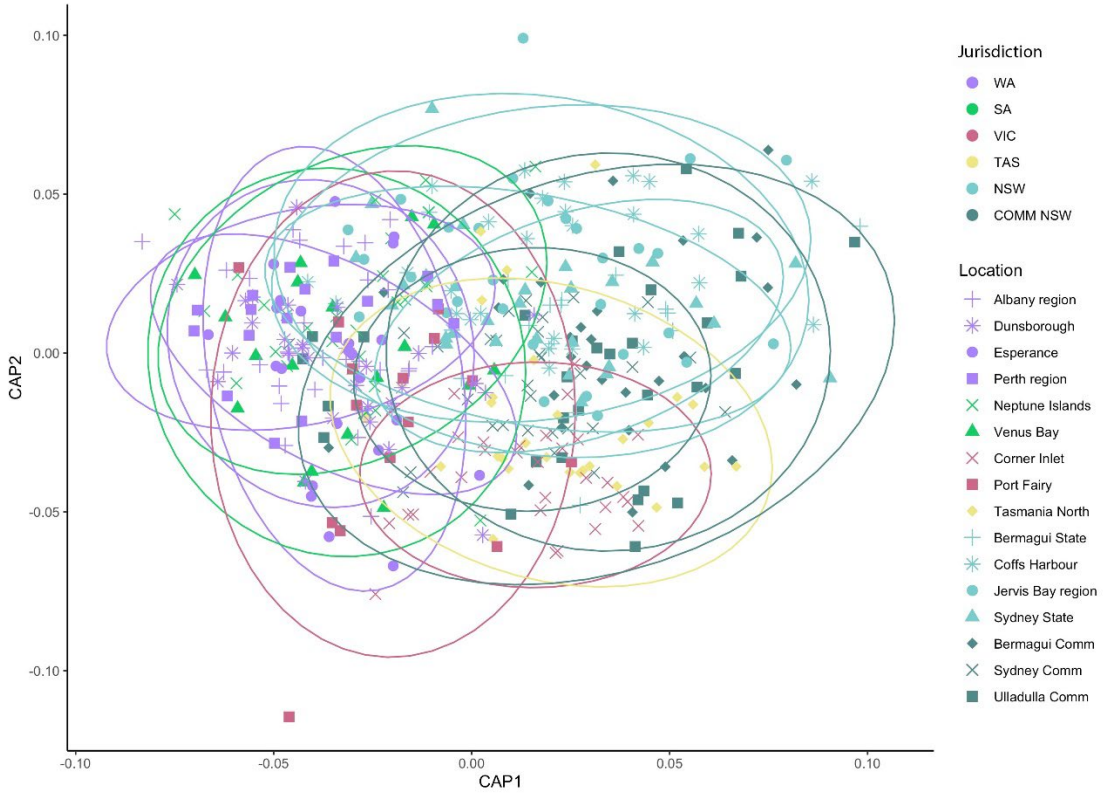

Figure 8. Ordination plot of the canonical analysis of principle coordinates (CAP) for wavelet coefficients comparing otolith shape of Silver Trevally across all sampling locations (overall correct classification 21 %). Also shown are 95 % confidence ellipses by location.

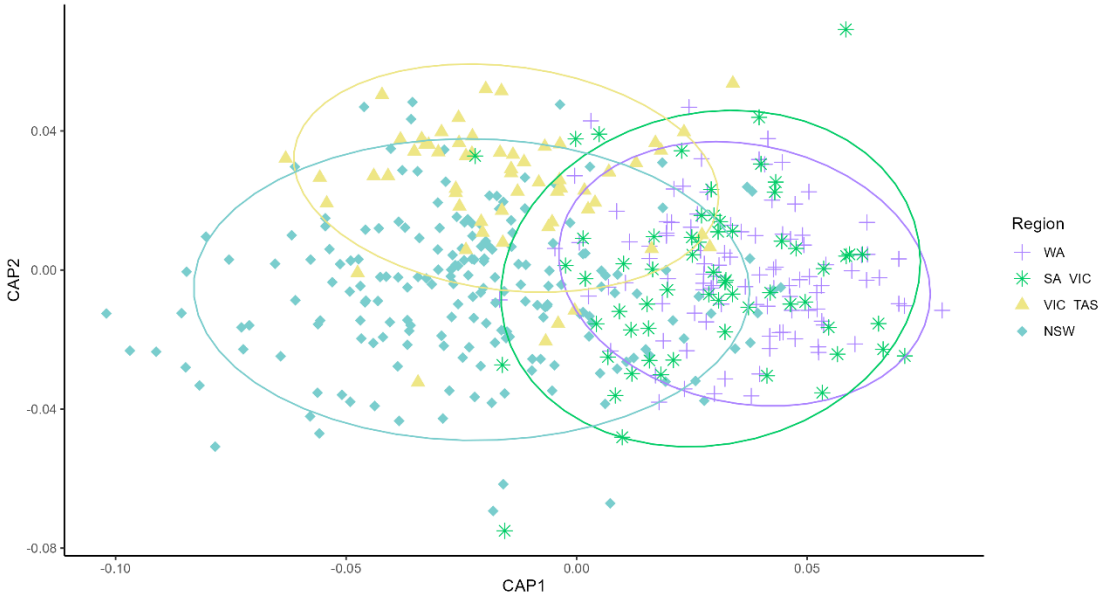

Figure 9. Example of ordination plot of the canonical analysis of principle coordinates (CAP) for otolith shape composition of Silver Trevally across pooling regions (overall correct classification 62 %). Groups shown are WA, SA and Western VIC (SA_VIC), Eastern VIC and TAS (VIC_TAS), and NSW including Commonwealth waters (NSW). Also shown are 95 % confidence ellipses by region.

Grouping adjacent locations within jurisdictions did not strongly increase overall classification accuracy (53 %), but trends in misclassification amplified the observation of trends in groupings among SA/western VIC and WA, against eastern VIC, TAS and NSW/Commonwealth. Silver Trevally from Commonwealth fisheries did not show a distinct otolith shape compared to others from NSW and other regions.

Table 5. Summary table of overall correct classifications (%) of individual Silver Trevally to collection location based on canonical analysis of principle coordinates (CAP) of otolith shape among individual locations, and main region comparisons by pooling locations within and among jurisdictions and broader regions.

Locations	**Overall classification success (%)**
All locations	**20.6**
States and Commonwealth	**42.3**
Jurisdictions	**53.3**
(WA - SA) Vs (VIC - TAS - NSW w/CmW)	**83.2**
(WA - SA - westVIC) Vs (eastVIC - TAS - NSW w/CmW)	**85.9**

However, it is important to further highlight the variation in shape between Port Fairy (VIC) and Corner Inlet (VIC), indicating reduced connectivity and increased structure in this region, where 12 of 15 samples from Western Victoria (i.e., Port Fairy) misclassified to the WA-SA cluster. When grouping samples from Port Fairy to the WA-SA region, overall classification increased to 86 % (with 90% and 84% correct classification per cluster – see appendices), suggesting the transition area between the two major groups occurs to the east of Port Fairy.

Overall, there are differences among combinations of locations (e.g., Pseudo-$F_{(4,567)}$ =6.8, P<0.001) but classification success was low or close to classifications expected by chance alone within jurisdictions and when pooling adjacent locations and regions (e.g., in WA (35 %), SA WA (45 %), VIC TAS (60 %), NSW all (25 %), NSW no CmW (42 %), NSW Vs NSW CmW (68%)).

Otolith chemistry

The elemental composition of otolith edges of Silver Trevally varied across locations and regions (Pseudo-$F_{(23,655)}$ = 16.7, P<0.001, Pseudo-$F_{(5,655)}$ = 28.5, P<0.001). Further analysis revealed there were variations across locations among the different elements analysed (e.g., Sr, Ba, Fig. 10).

Overall, the elemental signatures did not provide high discriminatory power at the broad national scale when analysing all collection locations together (42 % overall correct classification accuracy) (Fig. 11, Table 6). Nonetheless, there were individual locations with high and moderate classification accuracies, namely Coffs Harbour (NSW) with 84 %, followed by Jervis Bay (NSW 77 %), Corner Inlet (VIC 67 %) and Sydney State (NSW 67 %). Higher discrimination of key locations and trends in misclassification among regions suggest sub-structuring at finer scales (Fig. 11, Table 6). Overall, CAP analysis and sample distribution across the multivariate space highlight trends among jurisdictions and broader east to west regions that were further explored. Pooling SA and western VIC, and Tasmania with East Vic increased overall classification accuracy to 67%. At the national scale, a differentiation between WA, SA and western VIC versus eastern VIC, TAS and NSW is apparent, with each group attaining 73 % and 79 % classification accuracies (76 % overall correct classification) (Table 6, appendices).

Table 6. Summary table of overall correct classifications (%) of individual Silver Trevally to collection location based on canonical analysis of principle coordinates (CAP) of otolith chemistry among individual locations, and main region comparisons by pooling locations within and among jurisdictions and broader regions.

Locations	Overall classification success (%)
All locations	**42.3**
States and Commonwealth	**59.3**
Broad Region (WA, SA-VIC, VIC-TAS, NSW)	**67.2**
(WA - SA - westVIC) Vs (eastVIC - TAS - NSW)	**76.4**

Otolith chemistry for Silver Trevally showed regional differentiation among adjacent regions but also among locations at smaller spatial scales (Table 7). For instance, within NSW, Sydney, Coffs Harbour and Jervis Bay all had classification accuracies > 85%. An overall classification accuracy of 78 % was found when pooling all NSW state managed locations and all commonwealth waters (Fig. 12). For WA, overall classification accuracy also improved from 54 % (all locations within the state) to 77 % when combining samples collected from Perth (Metropolitan), nearby South-west and Albany [South Coast (West)] (Fig. 13).

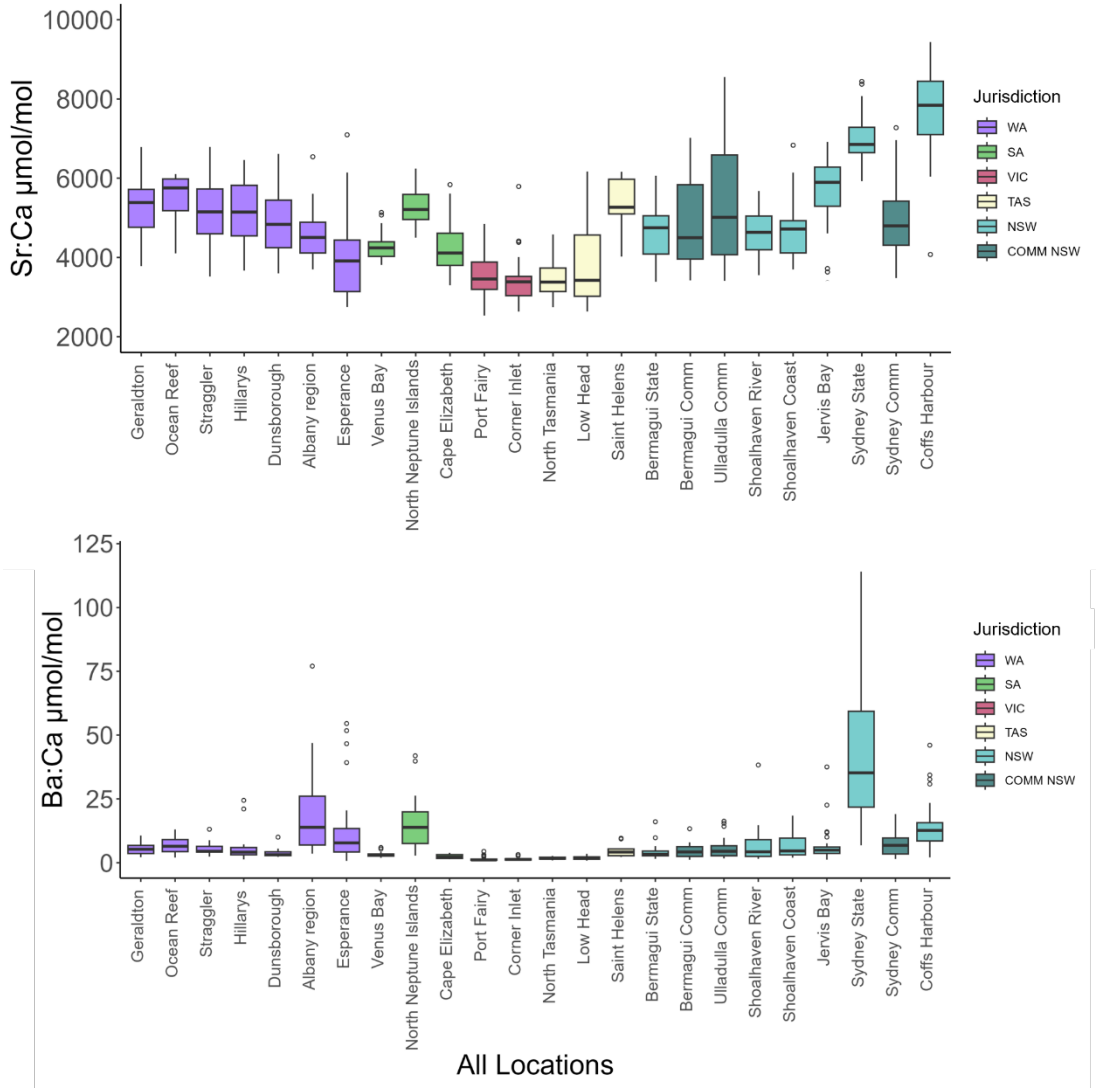

Figure 10. Sr:Ca (top) and Ba:Ca (below) concentration in otoliths of Silver Trevally collected across all sampling locations.

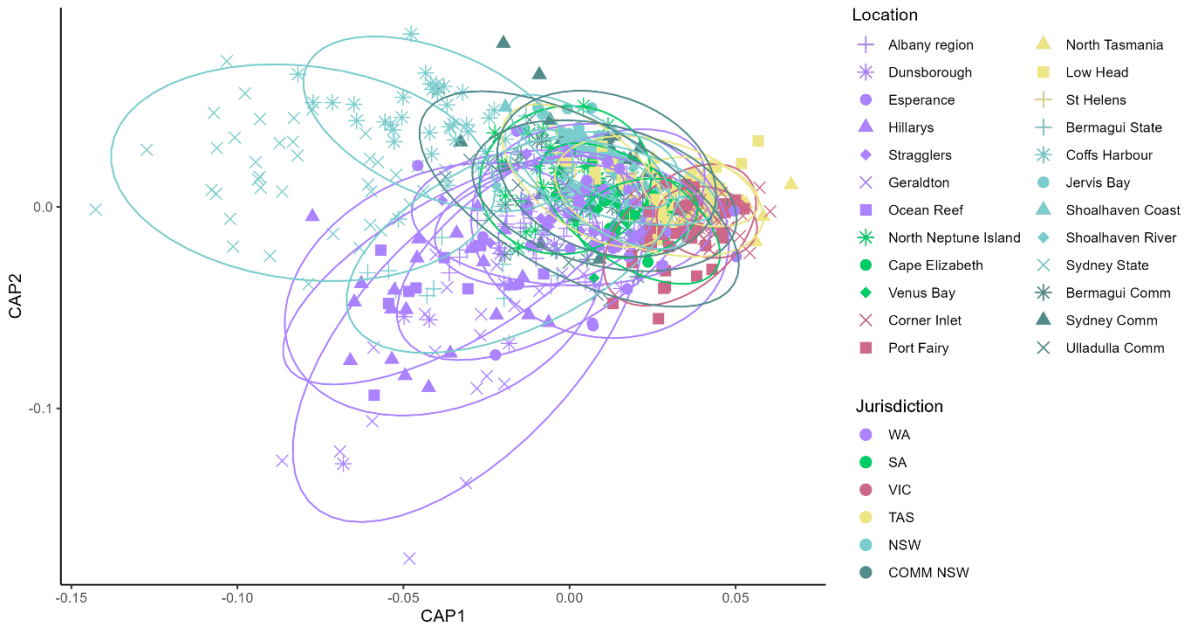

Figure 11. Ordination plot of the canonical analysis of principle coordinates (CAP) for otolith chemical composition of Silver Trevally across all locations (42% overall correct classification accuracy). Also shown are 95 % confidence ellipses by location.

Table 7. Summary table of overall correct classifications (%) of individual Silver Trevally to collection location based on canonical analysis of principle coordinates (CAP) of otolith chemistry to infer fine scale structuring.

Locations	**Overall classification success (%)**
Fine scale structuring (within jurisdiction)	
NSW and Commonwealth (all locations)	**68.6**
NSW Vs Commonwealth	**77.7**
TAS	**93.5**
VIC	**77.3**
SA	**75.3**
WA	**54.2**

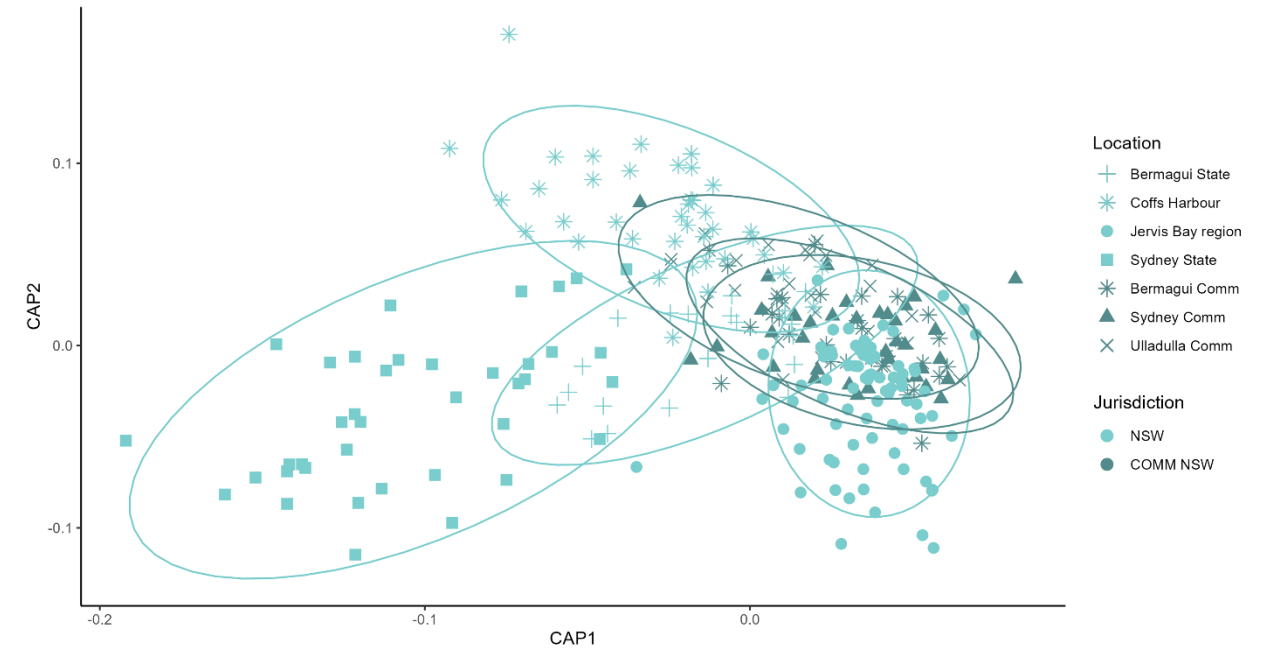

Figure 12. Ordination plot of the canonical analysis of principle coordinates (CAP) for otolith chemical composition of Silver Trevally from collection locations across NSW. Also shown are 95 % confidence ellipses by location.

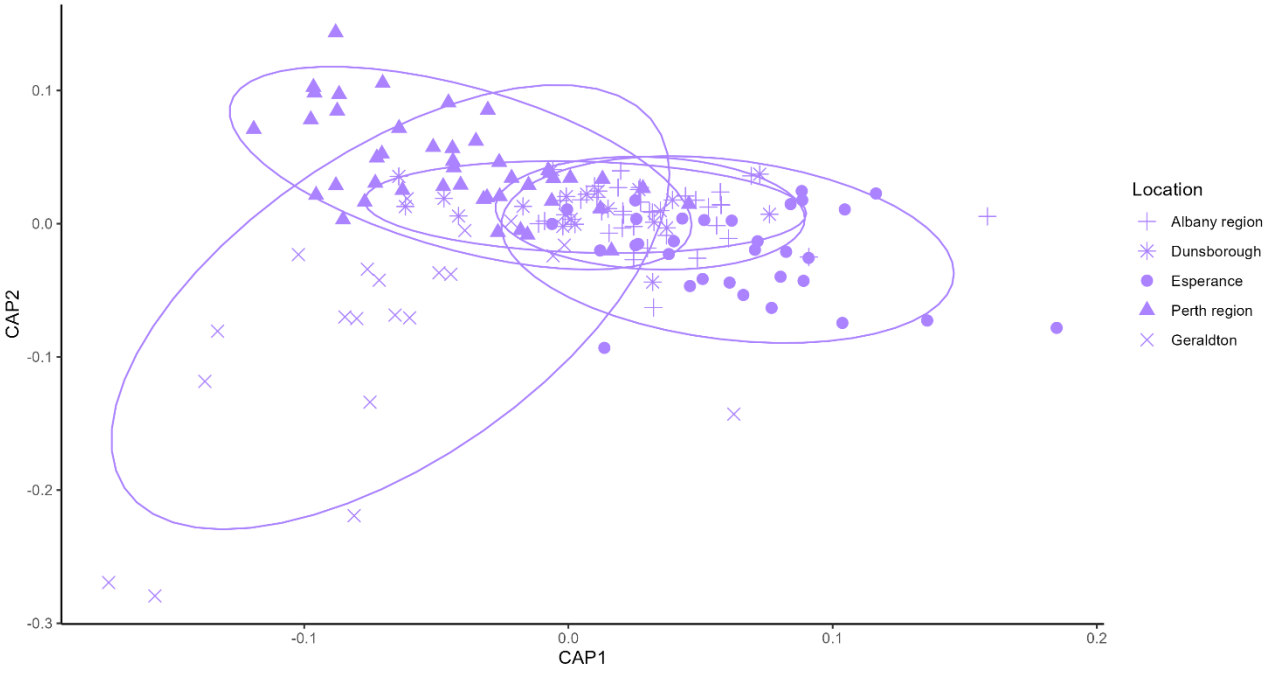

Figure 13. Ordination plot of the canonical analysis of principle coordinates (CAP) for otolith chemical composition of Silver Trevally from collection locations across WA. Also shown are 95 % confidence ellipses by location.

Integrating otolith shape and chemistry

Integrating both otolith markers did not bring added information on structuring when analysing all locations simultaneously (38.4 % overall classification). The results returned similar patterns at the regional and broad scale, delineating the strong east – west divide around VIC (88 % overall correct classification).

Genomic-based assessment

Methods

Sample collection

Samples of Silver Trevally from various locations across the Australian distribution were received by the Molecular Ecology lab., Flinders University (MELFU) (Fig. 14, Table 8). Samples were either preserved in vials with 100% ethanol or in Allflex® Tissue Sampling Unit (TSU) units.

Samples of Silver Trevally (n = 75) were also sourced from three regions (east, west, and north) in New Zealand (NZ) through a collaboration with researchers from the Victoria University of Wellington and Plant and Food Research. The NZ samples were used for an analytical comparison, particularly with the eastern Australian samples due to its closest geographic location and potential opportunities for intermixing due to current patterns in the Tasman Sea.

In addition, samples from 50 Sand Trevally *Pseudocaranx wrighti* were taken from Cockburn Sound in WA for future analyses that are not considered within the scope of this report.

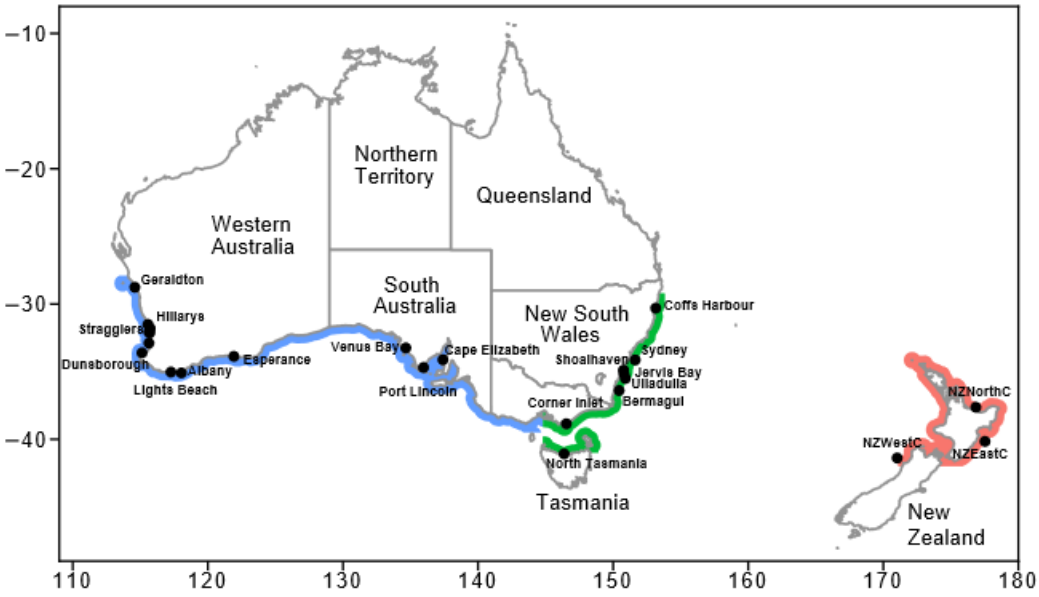

Figure 14. Map showing the locations of samples from Silver Trevally available for genomic analyses. Coloured coastlines represent the putative distribution of different genetic populations (i.e., stocks) identified in this study (details under Results).

Table 8. List of samples from Silver Trevally that were received, sequenced, and genotyped per region and locality. Note that the 50 fish from Cockburn Sound (WA) in italics were Sand Trevally *Pseudocaranx wrighti*.

Region	Locality	Received	Sent to Dart	Sequenced	Genotyped	Pop/Stock
New Zealand	NZEastC	24	24	24	24	New Zealand
New Zealand	NZNorhtC	24	24	24	24	New Zealand
New Zealand	NZWestC	27	27	27	27	New Zealand
NSW	Ballina	4	0	0	0	
NSW	Coffs Harbour	40	40	33	23	East Coast
Commonwealth	Com Sydney	80	40	40	40	East Coast
NSW	Sydney	40	40	2	0	
NSW	Shoalhaven	42	40	34	18	East Coast
NSW	Jervis Bay	40	40	20	9	East Coast
Commonwealth	Com Ulladulla	69	29	29	29	East Coast
Commonwealth	Com Bermagui	71	31	31	31	East Coast
NSW	Bermagui	23	22	5	0	
VIC	Corner Inlet	71	30	30	30	East Coast
VIC	Port Fairy	40	0	0	0	
TAS	Low Head	29	0	0	0	
TAS	St Helens	10	0	0	0	
TAS	North Tasmania	40	40	38	38	East Coast
SA	Cape Jervis	2	0	0	0	
SA	Cape Elizabeth	14	14	8	7	SouthWest Coast
SA	Port Lincoln	30	29	29	28	SouthWest Coast
SA	Venus Bay	32	29	27	18	SouthWest Coast
WA	Esperance	63	40	40	40	SouthWest Coast
WA	Albany	55	40	39	39	SouthWest Coast
WA	Lights Beach	4	4	4	4	SouthWest Coast
WA	Black Point	1	0	0	0	
WA	Gracetown	1	0	0	0	
WA	Cape Naturaliste	1	0	0	0	
WA	Dunsborough	39	38	38	38	SouthWest Coast
WA	Siesta Park	1	1	1	1	SouthWest Coast
WA	Busselton	1	0	0	0	
WA	Bunbury	3	0	0	0	
WA	Preston Beach	2	2	2	2	SouthWest Coast
WA	*Cockburn Sound*	*50*	*34*	*33*	*0*	
WA	Five Fathom Bank	1	0	0	0	
WA	Stragglers	10	10	10	10	SouthWest Coast
WA	Rottnest Island	8	8	8	8	SouthWest Coast
WA	Hillarys	27	25	24	23	SouthWest Coast
WA	Ocean Reef	11	9	9	9	SouthWest Coast
WA	Two Rocks	2	2	2	2	SouthWest Coast
WA	Geraldton	21	21	21	21	SouthWest Coast
	Total	1006	733	687	565	

Additional SNP filtering was done on the Silver Trevally samples as described in the General Methods section (Table 9).

Table 9. Additional filtering steps implemented at MELFU after DArTseq, showing the number of retained SNPs after each step for Silver Trevally.

Filtering criteria	Number of individuals	Number of SNPs
Dart raw SNPs	564	64,239
Missing data per individual <20%	543	64,239
Polymorphic SNPs	543	64,239
Reproducibility >= 0.99	543	48,015
Call rate per locus >= 0.8	543	29,824
MAF >= 3%	543	12,063
Depth of coverage > 10X < 100X	543	9,692
Out of HWE < 1 pops	543	2,675
One SNP per read	543	2,526

Results

SNP datasets of Silver Trevally

High quality genome-wide datasets containing 2,526 loci or SNPs were obtained for Silver Trevally after stringent filtering (Table 9). The Silver Trevally dataset is represented by 543 samples from 26 locations extending from Coffs Harbour in northern NSW to Geraldton in western WA (Fig. 14).

Genomic diversity in Silver Trevally

In general, similar levels of genomic diversity were observed for Silver Trevally across its range, with expected heterozygosity values ranging between 6 – 8 % (Table 10).

Table 10. Genomic diversity statistics for Silver Trevally at 26 localities and based on 2,526 SNPs. Number of individuals after filtering (N), number of SNPs after filtering (NL), proportion of polymorphic loci (% PL), observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (FIS).

Locality	N	NL	% PL	Ho	He	FIS
NZEastC	24	2,526	48.46	0.0661	0.066	0.0196
NZNorthC	24	2,526	48.73	0.0658	0.0667	0.0346
NZWestC	27	2,526	50.08	0.0662	0.0674	0.0359
Coffs Harbour	23	2,526	45.88	0.0564	0.0575	0.0403
Com Sydney	40	2,526	59.98	0.0616	0.0610	0.0030
Shoalhaven	18	2,526	42.40	0.0628	0.0637	0.0429
Jervis Bay	9	2,523	31.03	0.0614	0.0604	0.0437
Com Ulladulla	29	2,526	56.93	0.0603	0.0619	0.0422
Com Bermagui	31	2,526	54.20	0.0627	0.0614	−0.0046
Corner Inlet	30	2,526	54.67	0.0637	0.0628	0.0027
North Tasmania	38	2,526	58.39	0.061	0.0608	0.0097
Port Lincoln/Cape Elizabeth	35	2,526	53.21	0.0694	0.0688	0.0068
Venus Bay	18	2,526	49.11	0.0781	0.0779	0.0284
Esperance	40	2,525	59.34	0.0694	0.0676	−0.0131
Albany/Lights Beach	43	2,526	54.00	0.0692	0.0673	−0.0161
Dunsborough/Siesta P	39	2,526	54.49	0.0639	0.0633	0.0044
Preston Beach	2	2,526	12.07	0.0632	0.0482	0.0181
Stragglers/Rottnest I	18	2,526	45.79	0.0688	0.0663	−0.0094
Hillarys/Ocean Reef/Two R	34	2,513	55.97	0.0702	0.0688	−0.0059
Geraldton	21	2,526	44.97	0.0648	0.0635	0.0045

Genetic stock structure in Silver Trevally

Stock structure analyses of Silver Trevally based on PCA (Fig. 15) and Admixture (Fig. 16) suggest highly divergent groups among Australia's southern and western coasts (Spencer Gulf, SA, to Geraldton, WA), Australia's east coast (Coffs Harbour, NSW to Corner Inlet, VIC, including northern TAS), and NZ (east, north and west regions). These represent noticeably differentiated genetic stocks. Both analyses also suggest the potential rare occurrence of migrants between groups as exemplified by one individual sampled in Commonwealth waters off Ulladulla that clustered with the southern/western group (Figs. 15, 16).

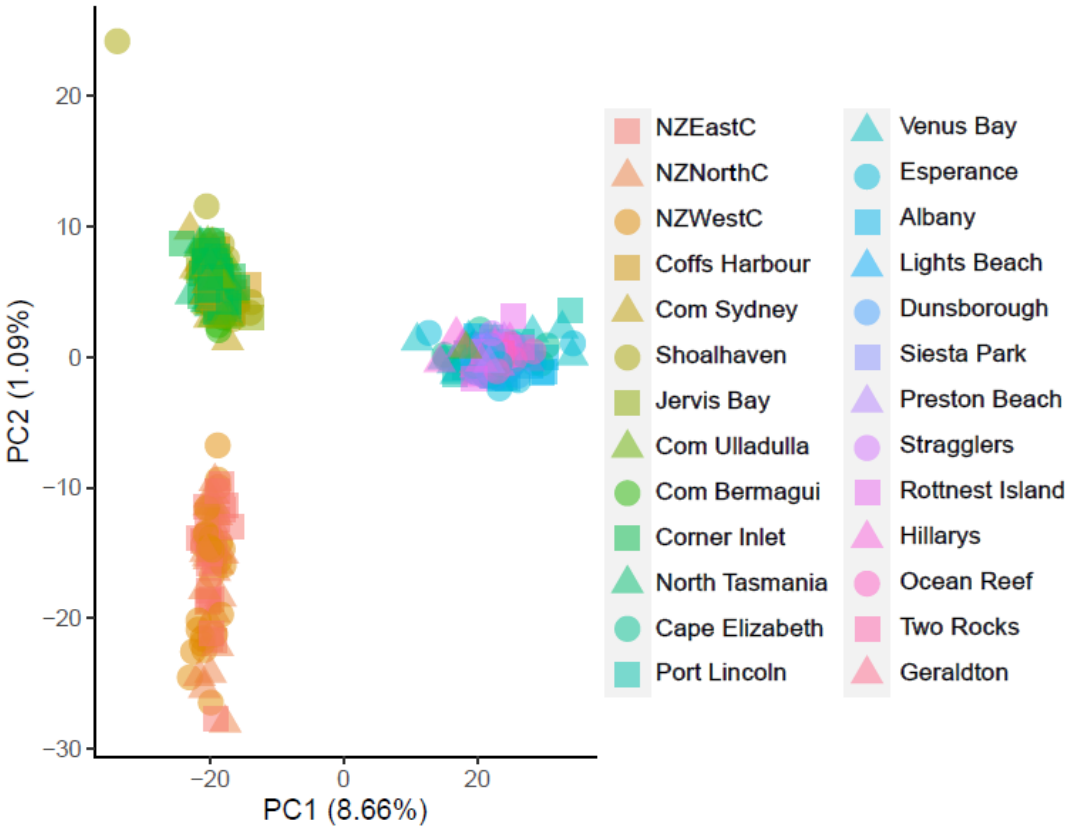

Figure 15. Principal Component Analysis (PCA) plot based on 2,526 SNPs illustrates the genomic variation among 543 samples of Silver Trevally from the coasts of Australia and New Zealand. The first two principal components explain 9.75% of the genomic variation and show three main genomic clusters.

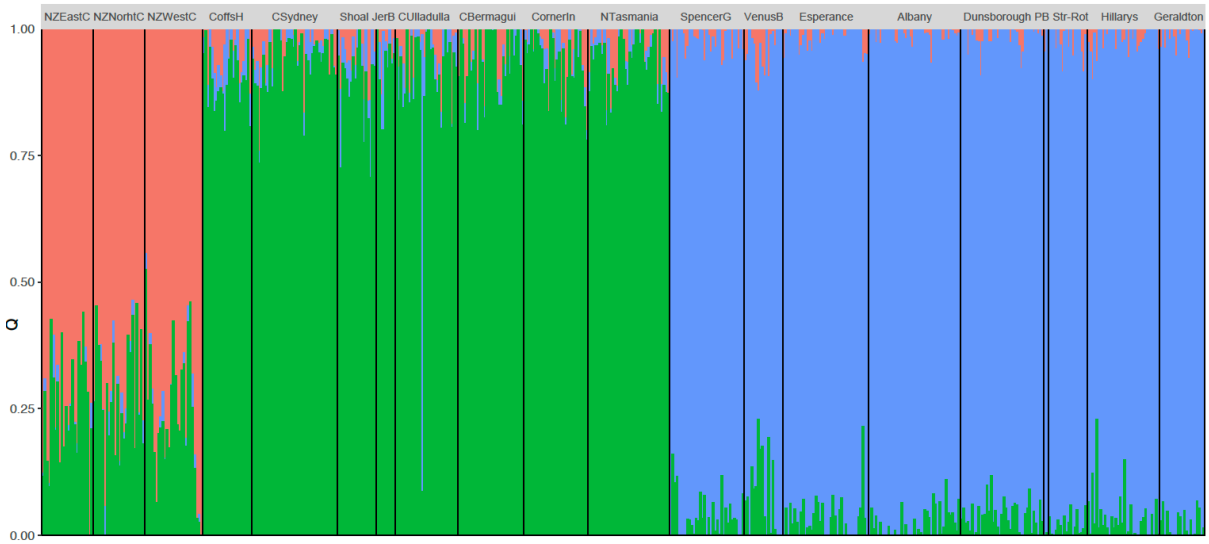

Figure 16. Admixture plot (K=3) based on 2,526 SNPs shows ancestry proportion for the 543 samples of Silver Trevally to each of the K genomic clusters.

Genetic differentiation in Silver Trevally

Fixation indices revealed low to negligible levels of genetic differentiation (i.e., high genetic connectivity) within each identified Silver Trevally group, and moderate to high differentiation between groups (i.e., low genetic connectivity), reinforcing the notion of two stocks of Silver Trevally present in Australia, and another in NZ (Fig. 17).

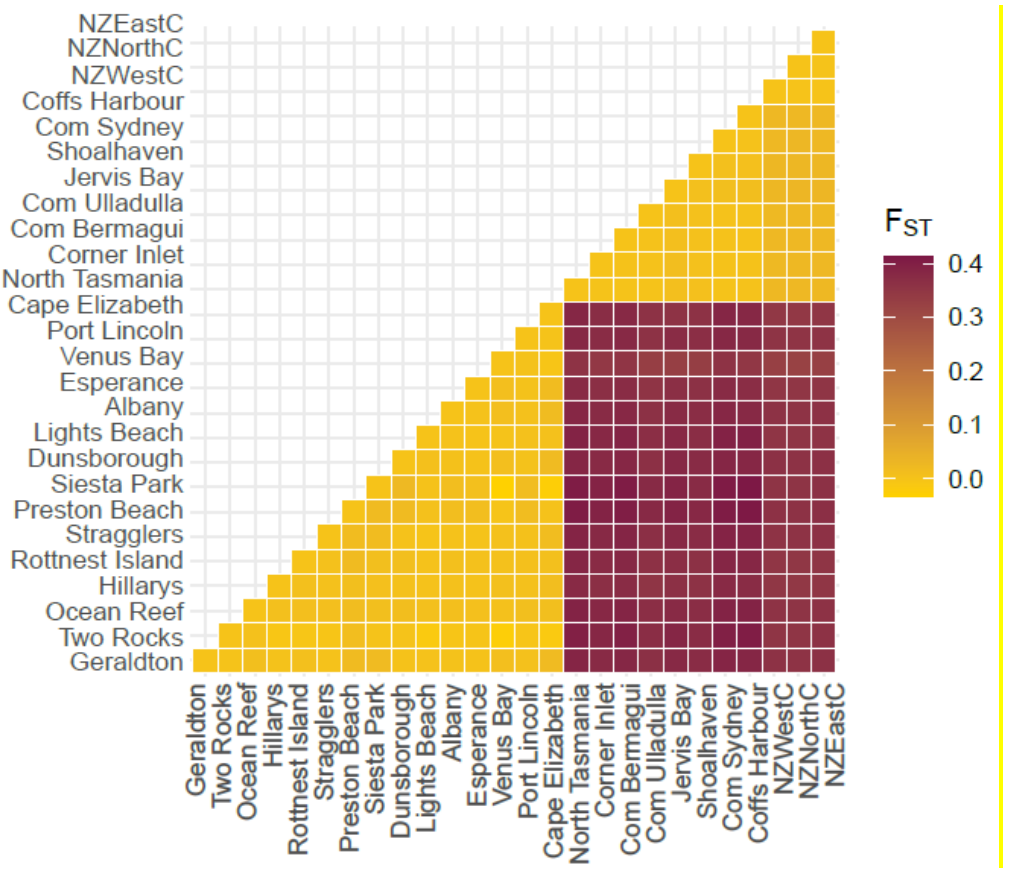

Figure 17. Heat map of pairwise FST calculated based on 2,526 SNPs and 543 samples of Silver Trevally. The heat map illustrates the genetic differentiation among 26 sampling localities across the coasts of Australia and New Zealand.

Isolation by distance in Silver Trevally

The analysis of isolation by distance, which tests for an association between geographic distance (coastal seascape distance in this case) and genetic distance, indicated an effect of geographic distance on genetic differentiation on a continental scale in Australia and NZ ($p < 0.001$; Fig. 18 a), but not within the three identified genetic stocks of Silver Trevally (Fig. 18 b, c, d).

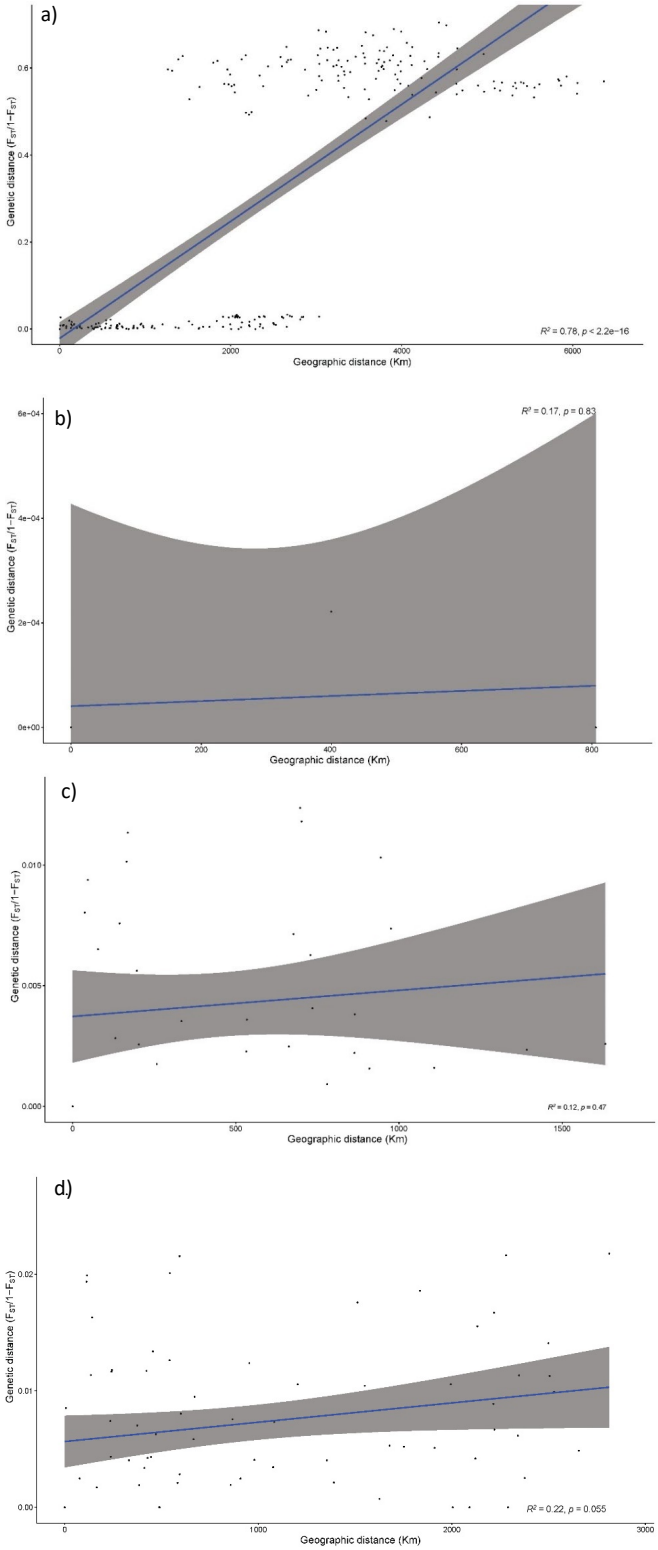

Figure 18. Mantel test scatter plots showing the correlation among genomic and geographic distances, and their respective 95 % confidence intervals, as a test for Isolation by Distance (IBD) on Silver Trevally samples from Australia and New Zealand. Test performed on: a) all 543 samples from 26 localities. b) 75 samples from three localities in New Zealand. c) 218 samples from eight localities across the East Coast of Australia. d) 250 samples from nine localities across the South and West Coasts of Australia.

Discussion

These results indicate that Australian Silver Trevally comprise two distinct biological stocks: a western biological stock that extends from Western Australia to western Victoria, and an eastern biological stock

extending from eastern Victoria to northern NSW, including north-eastern Tasmania and Commonwealth managed waters off New South Wales.

These western and eastern stocks divide somewhere between Port Fairy and Corner Inlet in Victoria. Unfortunately, genetic analyses of samples from western Victoria were not possible due to poor tissue preservation. However, results from the otolith shape and chemistry analyses grouped samples from western Victoria (Port Fairy) more successfully with the western stock of Silver Trevally. Low levels of genetic connectivity between the western and eastern stocks support a clear division somewhere between Spencer Gulf in South Australia, and north-eastern Tasmania and Corner Inlet, Victoria, and the otolith characteristics indicate it to be somewhere east of Port Fairy, Victoria.

The benefits of applying a multi-marker approach to this stock structure study are clear for Silver Trevally. The genomics assessment provides compelling evidence for discrete western and eastern biological stocks at a generational timescale. Genomics also shows high genetic connectivity within each stock indicating substantial gene flow. However, the otolith-based analyses were able to provide detail at the temporal scale of individual fish lifetimes. These otolith-based analyses provide evidence for potential fine-scale population structuring but will require a more targeted sampling effort and research into life-history and biology to be further resolved. Overall, while otolith shape analyses, that may indicate regional variability in growth rates, did not suggest population sub-structuring at smaller spatial scales, the otolith chemistry analyses did indicate potential regional and fine scale habitat use patterns. Differentiation in otolith chemistry between adjacent regions and among locations within regions suggest limited mixing of Silver Trevally over the few months captured by the laser ablations on the otolith edges. Noting, that the ability to discriminate among locations is based on variations in environmental conditions and fish habitat use patterns. This result is supported by the analyses of tag-recapture data off the coasts of Western Australia, New South Wales, and New Zealand that found limited movements of adults (James, 1980, Fairclough et al., 2011, Fowler et al., 2018).

The finding that Silver Trevally from New South Wales coastal locations could be classified with reasonable confidence from those sampled from offshore Commonwealth waters by their otolith edge chemistry may indicate different habitat use over recent timescales. Future work could consider the impact of seasonal or annual patterns and ontogeny on the distribution of individual Silver Trevally. Age-composition data from monitoring commercial fisheries off New South Wales show a large difference between fish captured in estuarine versus offshore fisheries. Silver Trevally sampled from estuaries tend to be less than five years of age, whereas those from offshore fisheries are generally aged three years and greater (to more than 20 years), suggesting an ontogenetic movement (Rowling and Raines, 2000, NSW DPIRD Unpublished data). This inshore/offshore distinction has also been observed in Western Australia, where larger/older individuals have been captured in deeper waters and have very different lengths at age (Farmer et al., 2005). However, it is unclear if this reflects a shift in habitat use during life or different assemblages of the same species.

Overall, results suggest that monitoring, assessment, and management at the scale of biological stocks of Silver Trevally is appropriate. Yet, otolith chemistry results indicate potential fine-scale population structure. To resolve this, further research in population dynamics and life-history parameters (e.g., growth rates, size and age at maturity) together with a more refined sampling design to understand stock structuring are necessary to determine implications for assessment and management. The potential for finer-scale structuring should be considered when designing programs to representatively sample the populations and the fisheries that exploit them.

There remain other areas of research that would benefit understanding of *Pseudocaranx spp*. in Australia. The present study has confirmed that fish landed as Silver Trevally nationally are *P. georgianus*, but several other similar species may co-occur. The samples of *P. wrighti* collected during the present study from Western Australia may increase understanding of relatedness with *P. georgianus*. Future genetic and morphometric work on *P. georgianus*, *P. wrighti, P. dinjerra* in Western Australia and *P. dentex* in Queensland and potentially Lord Howe Island would improve species identification across commercial and recreational fisheries.

Stock structure of Ocean Jackets *Nelusetta ayraud*

Introduction

Nelusetta ayraud, commonly called the ocean, yellow, or sand jacket and hereafter Ocean Jacket, is one of the largest species of monacanthid globally (Hutchins, 2000) attaining at least 790 mm TL (Kailola at el., 1993). The Ocean Jacket is endemic to Australia and supports substantial commercial and recreational fisheries nationally. The species is distributed from North West Cape in Western Australia around the south to Cape Moreton in Queensland, and there has been considerable research done about the species basic biology and fisheries off South Australia (Grove-Jones and Burnell, 1991, Lindholm, 1984) and New South Wales (Miller et al., 2010, Miller et al., 2013, Miller and Stewart, 2009). However, little is known of their biological stock structure. A pilot study by Dixon and Musa (1995) recommended that there was sufficient polymorphism for a future stock structure study of Ocean Jackets to be feasible, but could not discriminate significant differences between Coffs Harbour, Port Lincoln and the Great Australian Bight. Another pilot study reported some fine-scale (100s of km) population structuring off the coast of New South Wales, based on otolith chemistry but not shape (Gunton et al., 2023). Due to the lack of information on stock structure, Ocean Jackets are assessed and reported on for SAFS by jurisdiction (New South Wales, Victoria, Tasmania, South Australia) or management unit in the Commonwealth (Southeast Scalefish and Shark Fishery, Great Australian Bight Trawl Sector).

Otolith-based assessment

Results

Otolith shape

Wavelet shape reconstructions of whole Ocean Jacket otoliths generated 63 shape coefficients, but large variation of otolith characteristics among samples within locations are likely responsible for the limited success of this marker to infer patterns of population structure (Fig. 19, 20).

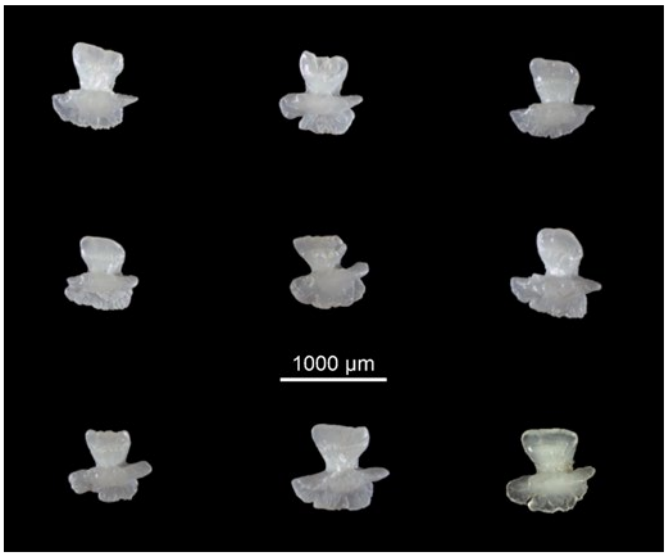

Figure 19. Images of a random selection of otoliths of Ocean Jackets from Coffin Bay (SA).

While observations of the mean otolith outlines resulted in significant variations in shape among locations (Pseudo-$F_{(10,278)}$ =17.1, P<0.001), this is not supported by subsequent CAP analysis, where overall classification to collection locations, jurisdictions, or pooled regions are low and not different from chance alone (13.2 %, 37.2 %, or 55 %, respectively) (Fig. 20, appendices). While PERMANOVA may detect group differences, it does not predict classification accuracy. Low classification success likely stems from complex

multivariate relationships that result in overlapping group distributions, hindering pattern identification for accurate predictions.

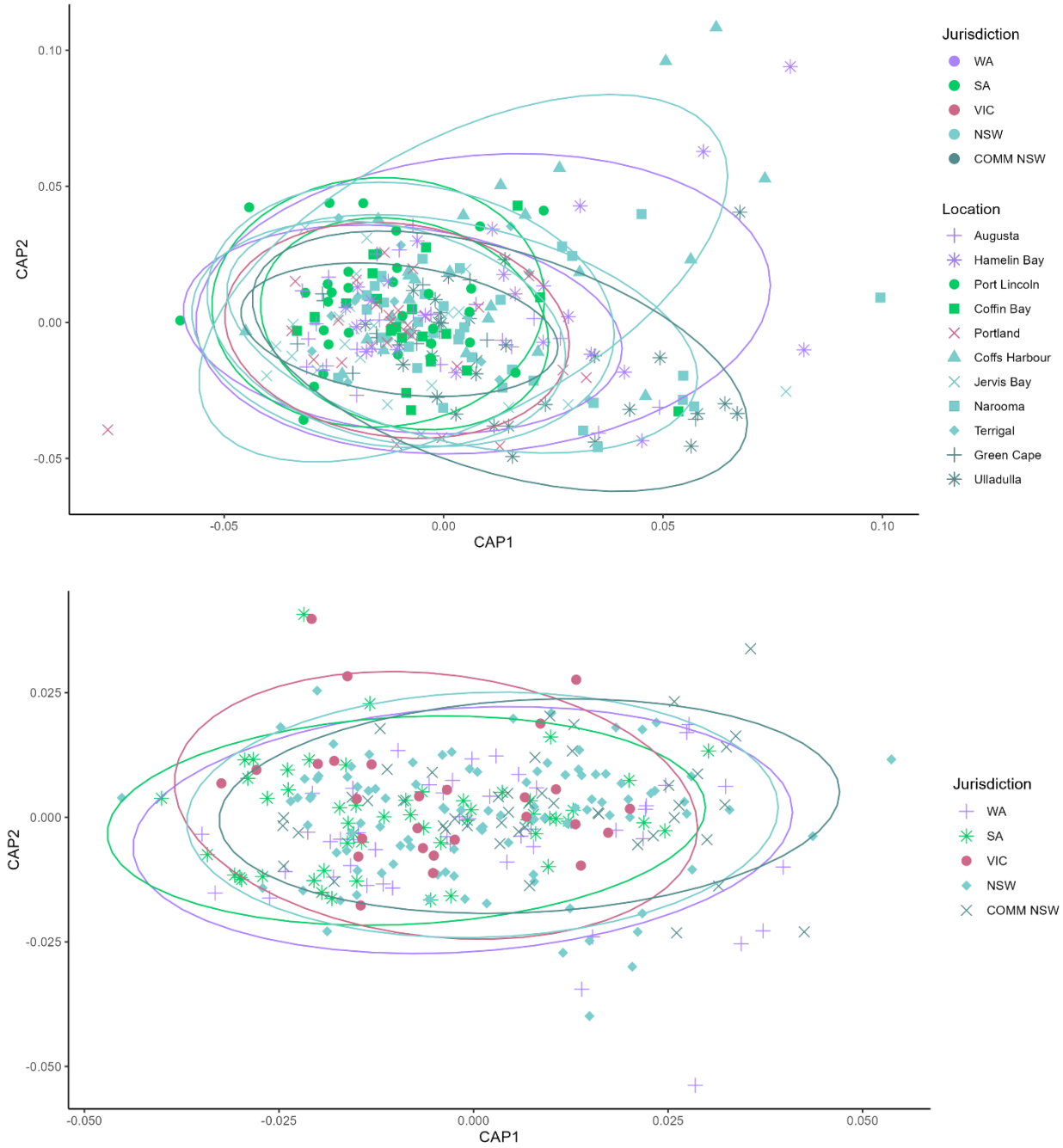

Figure 20. Ordination plot of the canonical analysis of principle coordinates (CAP) based on otolith shape showing no patterns or structuring for Ocean Jackets by location (top, overall classification accuracy 13 %) and jurisdiction (below, overall classification accuracy 37 %). Also shown are 95 % confidence ellipses by location (top) region (bottom).

Otolith chemistry

The elemental composition of otolith edges of Ocean Jackets varied across sites and regions (e.g., Pseudo-$F_{(8,245)}$ = 7.8, P<0.001, Pseudo-$F_{(4,245)}$ = 8.7 P<0.001), including among locations for different elements (e.g., Sr, Ba, Fig. 21).

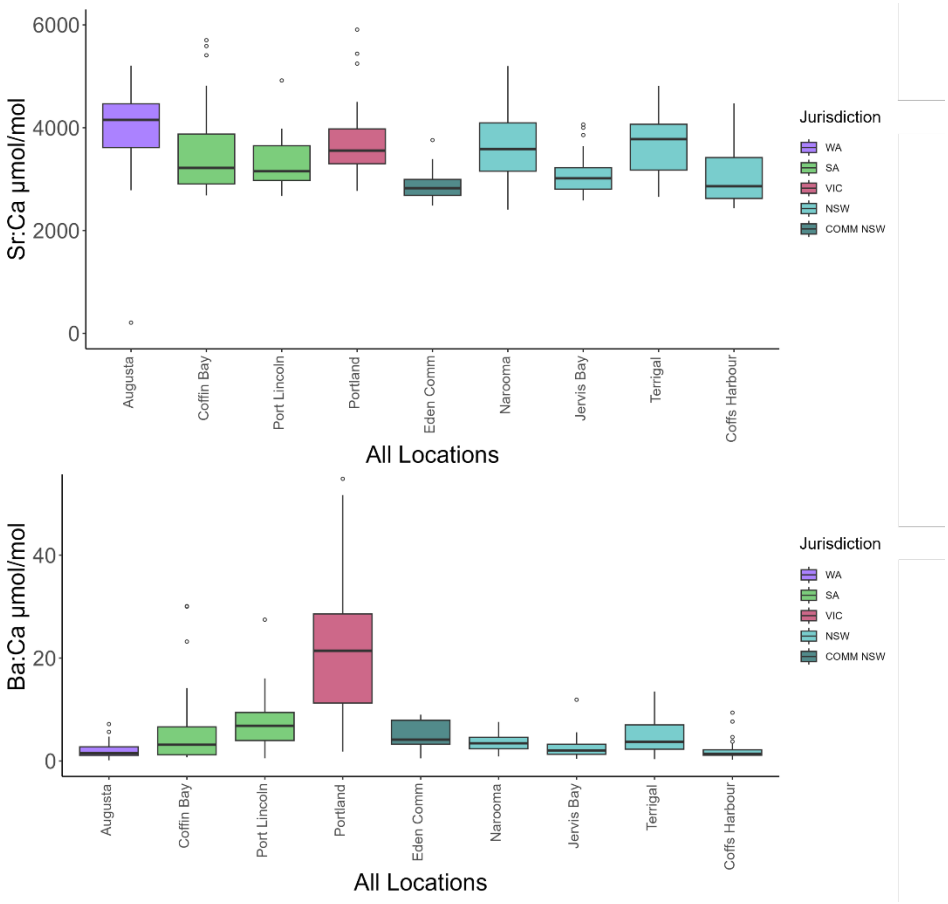

Figure 21. Sr:Ca (top) and Ba:Ca (below) concentrations in otoliths of Ocean Jackets collected across all sampling locations.

Based on all collection locations across Australia, otolith chemical composition showed moderate overall correct classification accuracy (56 %), though several locations had classification accuracies above or near 65 % (namely, NSW locations Terrigal 82 %, Narooma 79 %, Eden 70 %, Coffs Harbour 67 %, Portland, VIC 64 %) against others with 0 % (Coffin Bay, SA) (Table 11, Fig. 22). Similarly, broad-scale regional comparison by analysing jurisdiction and adjacent regions provides mixed results to infer stock structure but otolith chemistry composition revealed large-scale patterns that support western and eastern stocks (separating Western Australia, South Australia, and western Victoria from the east-coast sampling locations, overall correct classification 75 %) (Table 11, appendices).

Table 11. Summary table of overall correct classifications (%) of individual Ocean Jackets to collection location based on canonical analysis of principle coordinates (CAP) of otolith chemistry among individual locations, and main region comparisons by pooling locations within and among jurisdictions and broader regions.

Locations by chemistry	**Overall classification success (%)**
All locations	**55.7**
States and Commonwealth	**59.8**
Jurisdictions	**62.6**
WA SA wVIC	**57.3**
(WA – SA - wVIC) Vs (NSW)	**75.2**

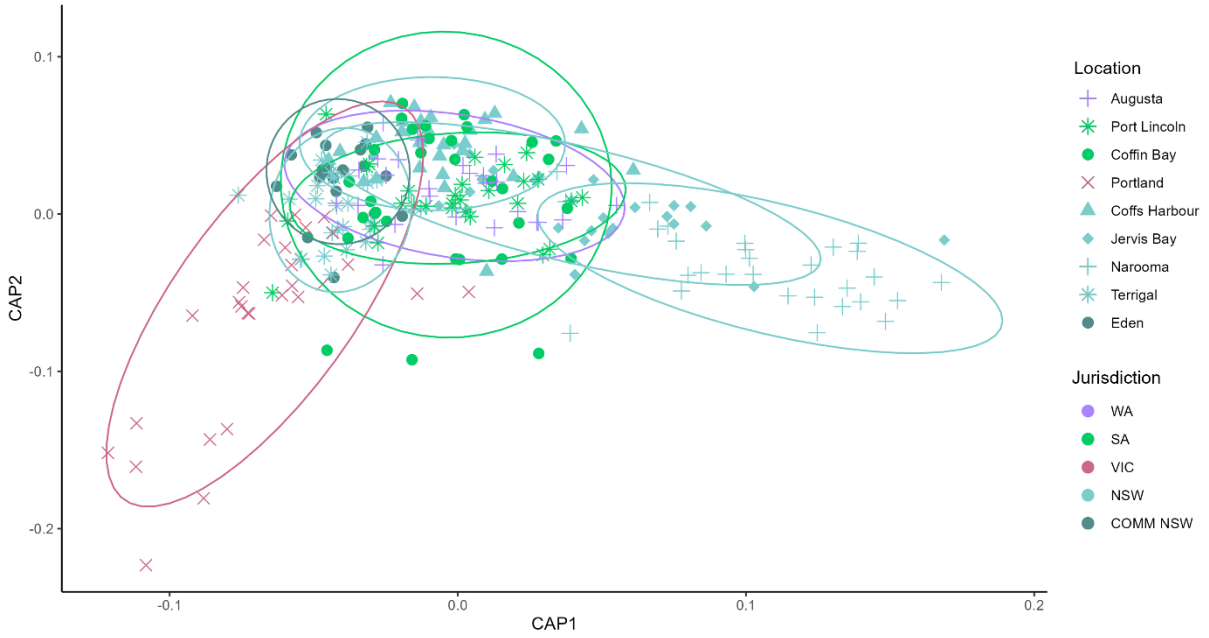

Figure 22. Ordination plot of the canonical analysis of principle coordinates (CAP) based on otolith chemistry of all locations for Ocean Jackets (overall correct classification 56 %). Also shown are 95 % confidence ellipses by location.

Further analysis suggests some habitat use patterns and potential fine-scale structuring within NSW, with 80% overall correct classifications per location, or 84 % and 95 % when pooling locations across the latitudinal gradient (North NSW – Coffs Harbour, Mid NSW – Terrigal, Lower NSW – Eden, Narooma, Jervis Bay) or state waters against Eden (Commonwealth) (Table 12, Fig. 23).

Table 12. Summary table of overall correct classifications (%) of individual Ocean Jackets to collection location based on canonical analysis of principle coordinates (CAP) of otolith chemistry to infer fine scale structuring.

Locations by chemistry	**Overall classification success (%)**
Fine scale structuring (within jurisdiction)	
SA	**66.7**
NSW jurisdiction (all locations)	**80.2**
NSW (regional pools)	**83.9**

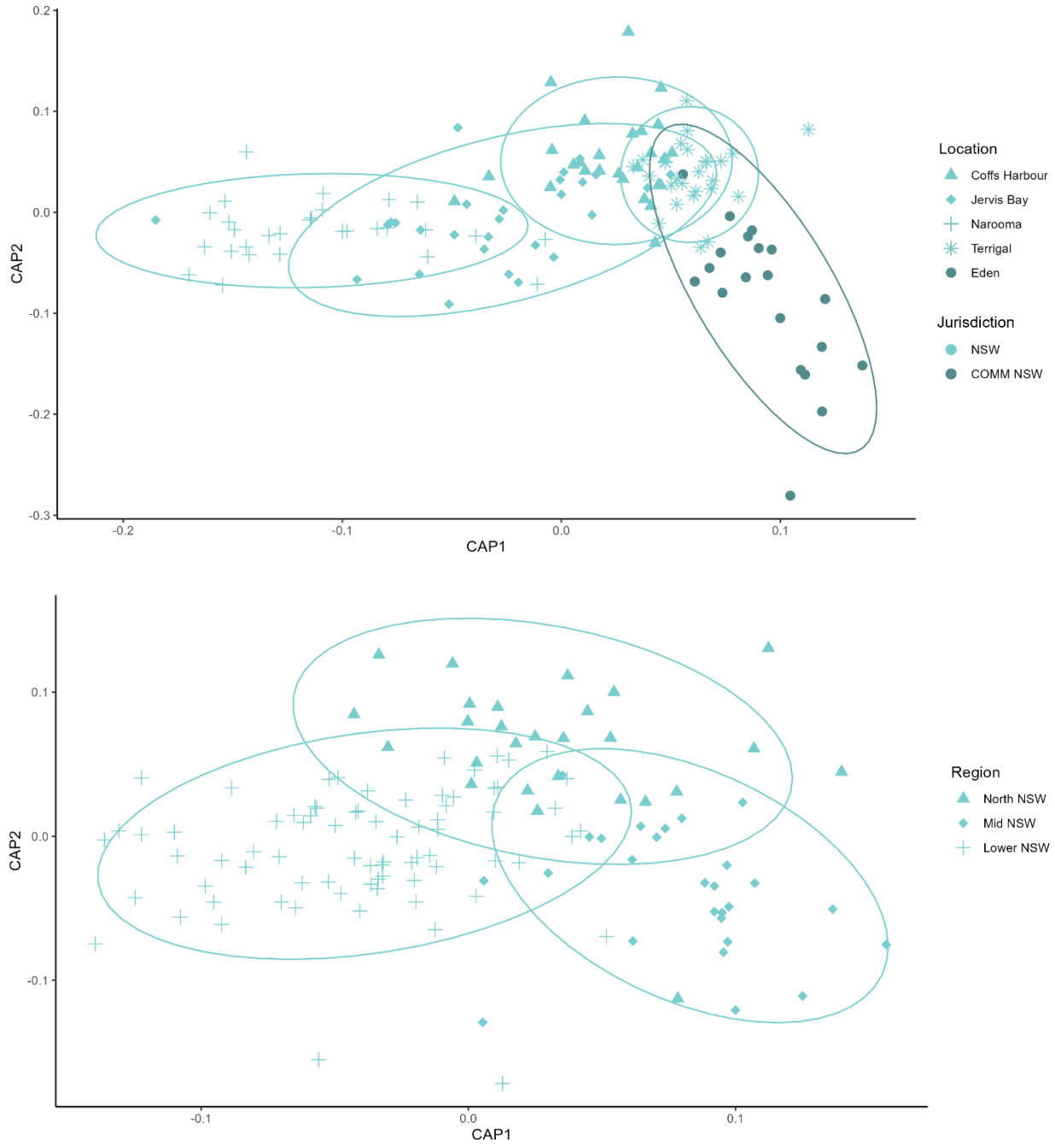

Figure 23. Ordination plot of the canonical analysis of principle coordinates (CAP) based on otolith chemistry of Ocean Jackets from NSW based on all locations (top, overall classification accuracy 80 %) and on latitudinal regional groupings (bottom, overall classification accuracy 84 %). Also shown are 95 % confidence ellipses by location (top) region (bottom).

Genomic-based assessment

Methods

Samples of Ocean Jackets from various locations across the Australian distribution were received by the Molecular Ecology lab., Flinders University (MELFU) (Fig. 24, Table 13). Samples were either preserved in vials with 100% ethanol or in Allflex® Tissue Sampling Unit (TSU) units.

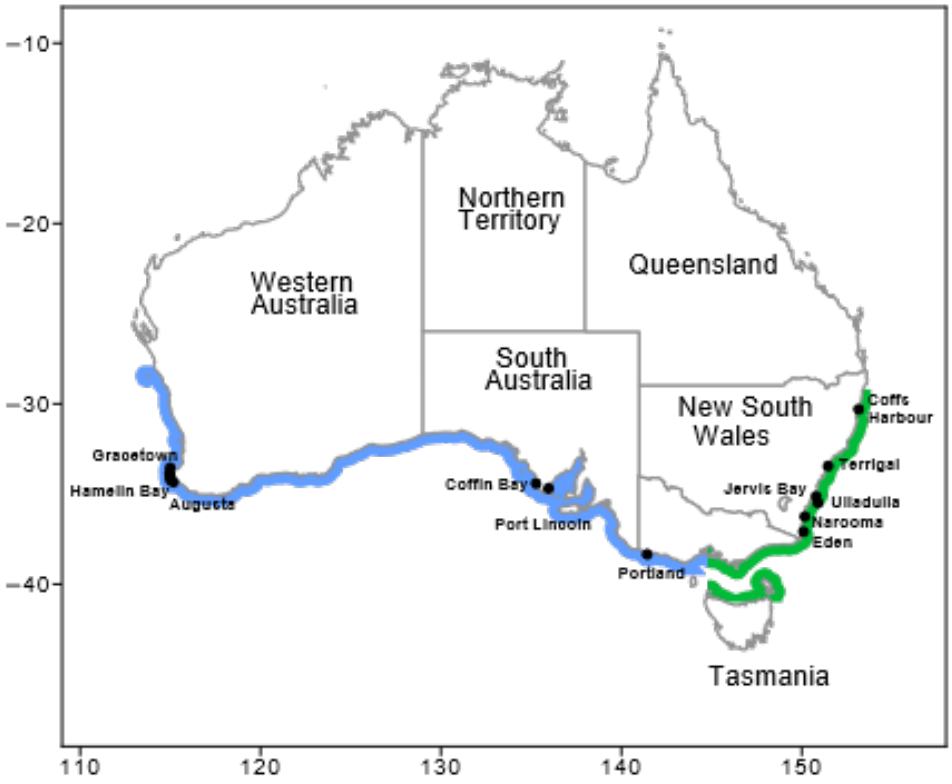

Figure 24. Map showing the locations of samples from Ocean Jackets available for genomic analyses. Coloured coastlines represent the putative distribution of different genetic populations (i.e., stocks) identified in this study (details under Results).

Table 13. List of samples from Ocean Jackets that were received, sequenced, and genotyped per region and locality.

Region	Locality	Received	Sent to DART	Sequenced	Genotyped	Pop/Stock
NSW	Ballina	1	0	0	0	
NSW	Coffs Harbour	40	40	40	31	East Coast
NSW	Terrigal	39	36	35	33	East Coast
NSW	Jervis Bay	40	35	35	35	East Coast
Commonwealth	Com Ulladulla	60	35	35	33	East Coast
NSW	Narooma	40	35	35	35	East Coast
Commonwealth	Com Eden	40	35	35	24	East Coast
Commonwealth	Greencape	20	0	0	0	East Coast
VIC	Portland	30	30	29	39	East Coast
SA	Coffin Bay	46	20	15	8	SouthWest
SA	Port Lincoln	38	38	36	35	SouthWest
WA	Augusta	32	32	32	31	SouthWest
WA	Hamelin Bay	29	29	27	27	SouthWest
WA	Margaret River	7	7	7	7	SouthWest
WA	Gracetown	3	3	3	3	SouthWest
	Total	465	375	364	341	

Additional SNP filtering was done on the Ocean Jacket samples as described in the General Methods section (Table 14).

Table 14. Additional filtering steps implemented at MELFU after DArTseq, showing the number of retained SNPs after each step for Ocean Jackets.

Filtering criteria	Number of individuals	Number of SNPs
Dart raw SNPs	365	33,065
Missing data per individual <20%	336	64,239
Polymorphic SNPs	336	32,693
Reproducibility >= 0.99	336	23,932
Call rate per locus >= 0.8	336	13,999
MAF >= 1%	336	7,345
Depth of coverage > 5X < 100X	336	7,159
Out of HWE < 1 pops	336	5,371
One SNP per read	336	4,869
Individual similarity >80%	331	4,869
Missing data individual per locality <20%	328	4,869

Results

SNP datasets of Ocean Jackets

High quality genome-wide datasets containing 4,869 loci or SNPs were obtained for Ocean Jacket, after stringent filtering (Table 14). The Ocean Jacket dataset is represented by 328 Ocean Jackets from 12 locations extending from Coffs Harbour in northern NSW to Cape Naturaliste (i.e., considered as Capes for samples collected between Cape Leeuwin and Cape Naturaliste) (Fig. 24).

Genomic diversity in Ocean Jackets

There were marked differences in the levels of genomic diversity between the two main Australian regions where Ocean Jackets are found, with southern Australian localities displaying higher levels of expected heterozygosity (14–17 %) compared to localities in eastern Australia (4–6 %) (Table 15).

Table 15. Genomic diversity statistics for Ocean Jackets at 12 localities and based on 4,869 SNPs. Number of individuals after filtering (N), number of SNPs after filtering (NL), proportion of polymorphic loci (% PL), observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (FIS).

Locality	N	NL	% PL	Ho	He	FIS
Coffs Harbour	31	4,869	41.45	0.0357	0.0358	0.0193
Terrigal	33	4,869	44.12	0.0408	0.0404	0.0055
Jervis Bay	35	4,869	41.53	0.0371	0.0369	0.0089
Com Ulladulla	33	4,869	52.52	0.0582	0.0533	-0.0734
Narooma	35	4,869	43.62	0.0347	0.0357	0.0427
Eden	8	4,867	24.33	0.0427	0.0435	0.0848
Com Eden	16	4,869	40.95	0.0596	0.0553	-0.0423
Portland	29	4,869	87.76	0.1663	0.1653	0.0115
Coffin Bay	5	4,867	39.94	0.1353	0.1102	-0.0996
Port Lincoln	35	4,869	88.66	0.1567	0.1614	0.0431
Augusta	31	4,869	87.27	0.1316	0.1516	0.1468
Cape	37	4,869	89.98	0.1334	0.1529	0.1396

Genetic stock structure in Ocean Jackets

Stock structure analyses of Ocean Jackets based on PCA (Fig. 25) and Admixture (Fig. 26) suggest two highly differentiated groups between Australia's east coast (Coffs Harbour to Eden, NSW) and southern and western coasts (Portland, VIC to the Capes, WA), with a greater spread (PCA) and mixed ancestries (Admixture) in the latter group.

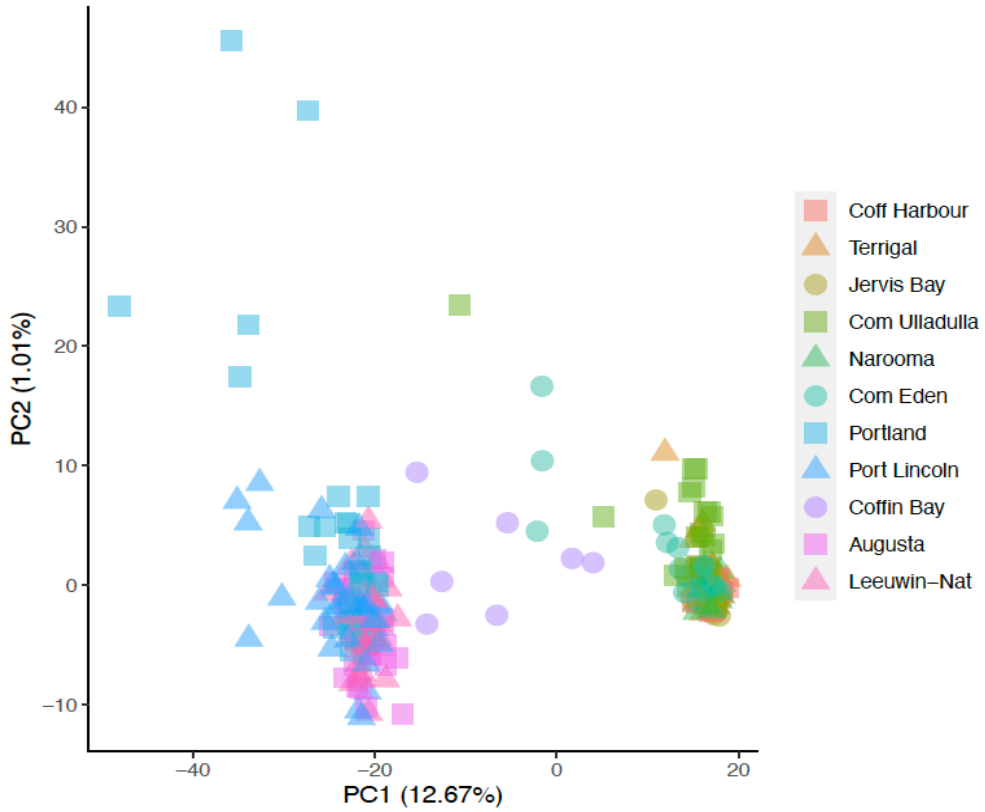

Figure 25. Principal Component Analysis (PCA) plot based on 4,869 SNPs illustrates the genomic variation among 328 samples of Ocean Jacket from the coast of Australia. The first two principal components explain 9.75 % of the genomic variation and show two main genomic clusters.

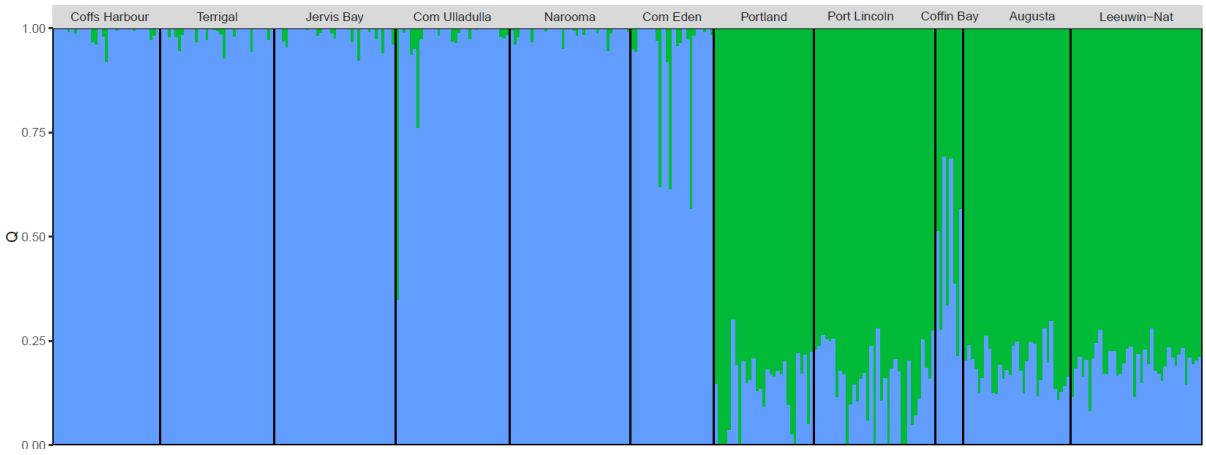

Figure 26. Admixture plot (K=2) based on 4,869 SNPs shows ancestry proportion for the 328 samples of Ocean Jacket to each of the K genomic clusters.

Genetic differentiation in Ocean Jackets

Similar to the Silver Trevally, fixation indices for Ocean Jackets revealed low to negligible levels of genetic differentiation (i.e., high genetic connectivity) within each identified group, and moderate differentiation between groups (i.e., low genetic connectivity) (Fig. 27).

Isolation by distance in Ocean Jackets

Isolation-by-distance tests were only significant ($p < 0.05$) when testing was done for the whole dataset, but not when testing was done for each identified stock separately (Fig. 28 a, b, c).

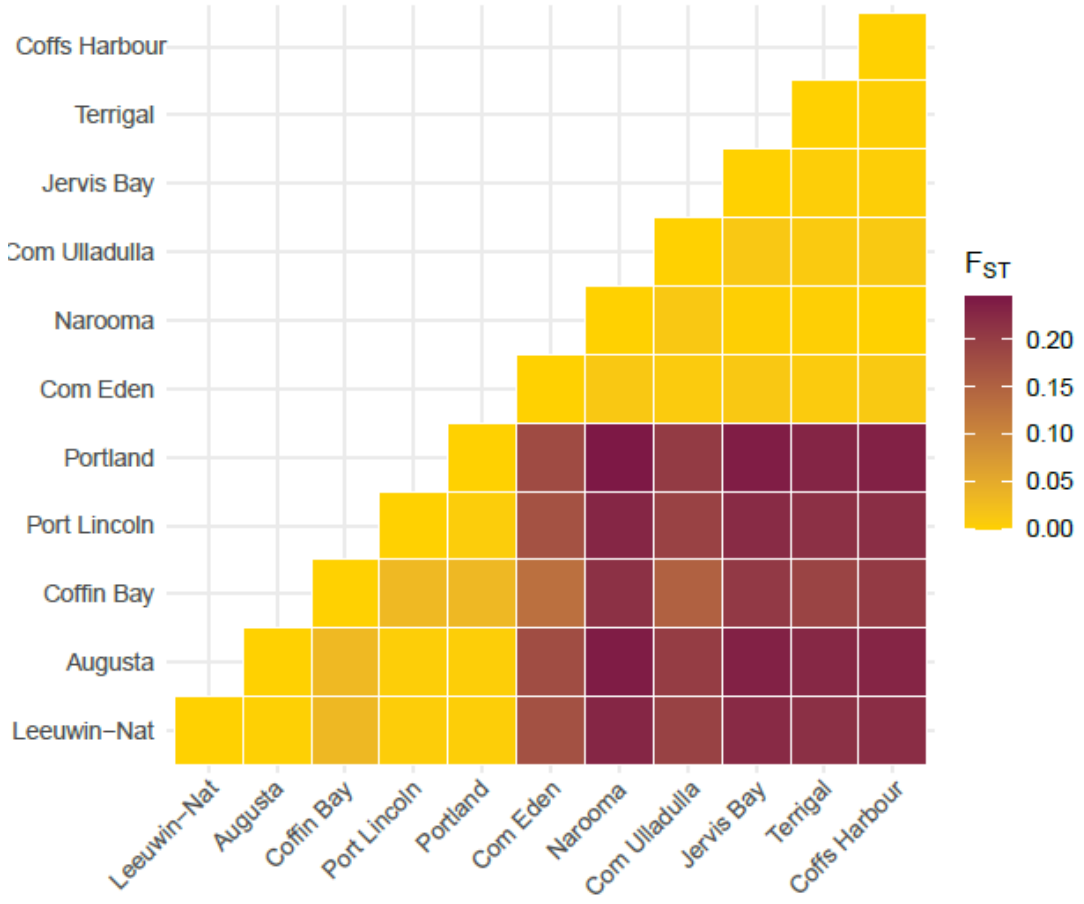

Figure 27. Heat map of pairwise FST calculated based on 4,869 SNPs and 328 samples of Ocean Jacket. The heat map illustrates the genetic differentiation among 11 sampling localities across the coast of Australia.

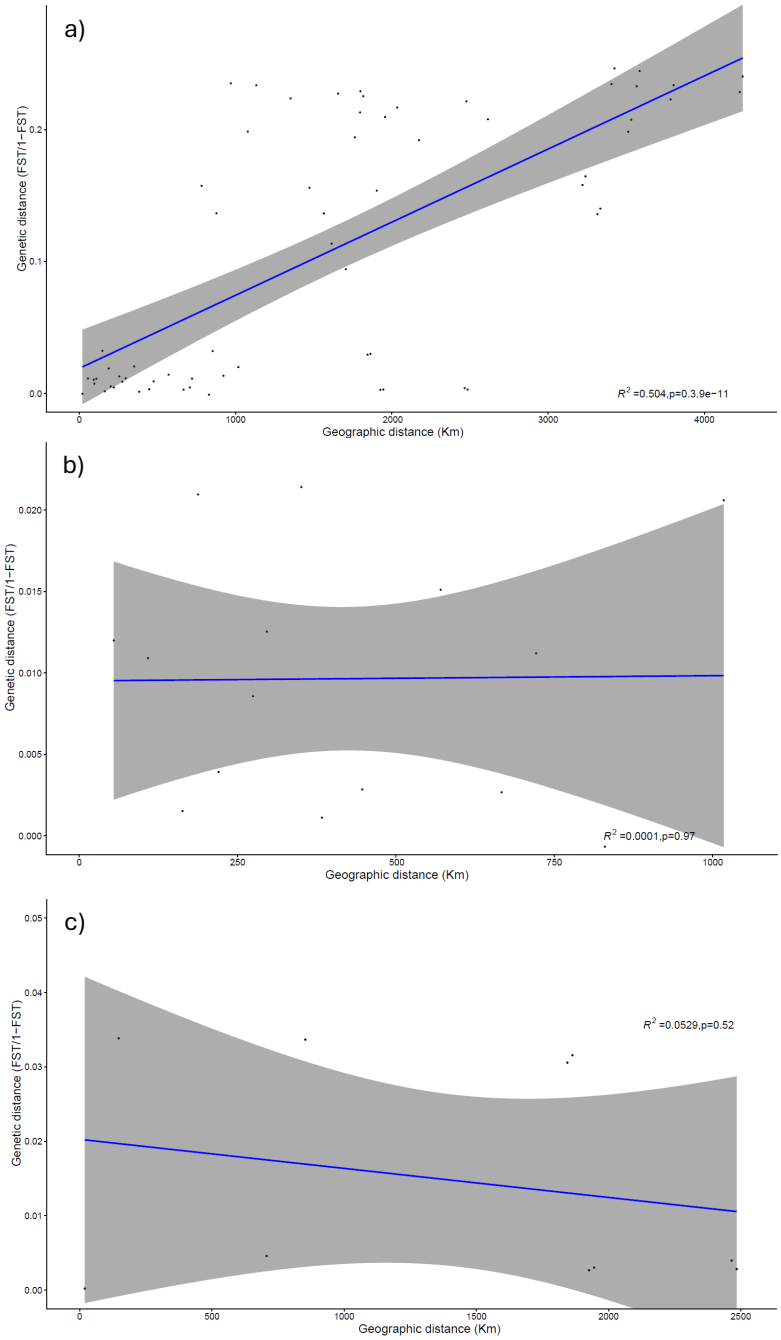

Figure 28. Mantel test scatter plots showing the correlation among genomic and geographic distances, and their respective 95 % confidence intervals, as a test for Isolation by Distance (IBD) on Ocean Jacket samples from Australia. Test performed on: a) all 328 samples from 12 localities. b) 191 samples from six localities across the East Coast of Australia. c) 135 samples from five localities across the South and West Costs of Australia.

Discussion

Similar to the findings for Silver Trevally, Ocean Jackets comprise two distinct biological stocks: a western biological stock that extends from Western Australia along the southern Australian coastline to western Victoria, and an eastern biological stock extending from northern New South Wales south along the east-coast, including Commonwealth managed waters (see Fig. 24). The boundary between these western and eastern biological stocks is somewhere east of Portland and west of Eden, and the ranges portrayed in Fig. 24 should be considered as coarse estimates.

Unfortunately, Ocean Jackets were not observed in landings at Lakes Entrance from Commonwealth endorsed vessels during the project, nor from northern Tasmanian waters where they are reported to be quite

rare and not targeted. However, given the influence of the southerly flowing Eastern Australian Current and the conceptual model of Ocean Jacket behaviour off eastern Australia (see below), it is likely that Ocean Jackets off eastern Victoria and northern Tasmania would be part of the eastern biological stock. Similarly in the west, Ocean Jackets are reported to occur as far north as North West Cape (latitude 21.8° S) but our sampling did not extend beyond the south-west corner of Western Australia. There is no evidence to suggest whether Ocean Jackets north of our sampling in Western Australia should be considered part of the western biological stock, but this is unlikely to impact fisheries assessment or management due to their relative rarity and low catches in those areas.

The genomics assessment showed a low degree of genetic connectivity between the western and eastern stocks of Ocean Jackets, indicative of isolated populations. However, within each biological stock genetic differentiation was extremely low, particularly within the eastern biological stock, indicative of well-mixed populations. Interestingly, genetic heterozygosity was greater within the western biological stock which may be associated with a larger population size than the eastern biological stock, which has historically supported greater harvests.

The lack of detectable population structuring in Ocean Jackets using otolith shape analyses might be due to the very small size (~1 mm) and inconsistent shape of their otoliths (Fig. 19). This is supported by a previous study that found no significant variation in otolith shape among Ocean Jackets from three locations and with fish size in NSW (Gunton et al., 2023). Despite their small size and inconsistent shape, otoliths of Ocean Jackets are useful for age estimation (Miller et al., 2010) and chemical analyses (Gunton et al., 2023). The present study of otolith chemistry composition revealed large-scale patterns that are consistent with the genomics of western and eastern stocks, separating Western Australia, South Australia, and western Victoria from the east-coast sampling locations. High classification accuracies based on otolith chemistry for some locations may infer potential finer-scale population structuring and/or habitat use. This was apparent for New South Wales Ocean Jackets where regional trends across a latitude gradient were evident, including for Commonwealth samples from more southern locations (Eden) relative to the remaining state samples (Narooma, Jervis Bay, Terrigal and Coffs Harbour). This may reflect differing water masses, and that fish were under similar environmental conditions per location over the few months the analysed chemical signal encompasses. Gunton et al. (2023) reported variations in otolith-edge chemistry with latitude but similar inconsistent classification accuracies (ranging between 30 and 71 % per location), with the southern site in that study showing the highest discrimination (i.e., Kiama), and suggested this may have been due to small-scale variations in water chemistry and environmental conditions. Distinction between NSW and Commonwealth jurisdictions in the current study may also have resulted from ontogenetic variation in otolith chemistry combined with spatial segregation of age-classes. New South Wales and Commonwealth fishery jurisdictions are complex and delineated by distance offshore and latitude. An offshore and northerly ontogenetic movement has been hypothesised for *N. ayraud* based on observations of juveniles within estuaries and larger, older individuals offshore (see below, Miller and Stewart, 2013). Gunton et al. (2023) also found that both Sr and Ba varied with age in *N. ayraud*. To further resolve this, a more constrained sampling strategy, targeting similar-sized individuals and cohorts is required. This will also allow for additional in-depth assessment of life-history parameters.

The finding that Ocean Jackets off eastern Australia represent a well-mixed genetic stock assists in developing a conceptual model of the species' life-history. It is known that Ocean Jackets form large spawning aggregations off northern New South Wales during July and August (Miller et al., 2013), with no substantial ovarian development observed in southern areas. Eggs and larvae are dispersed by the predominantly southerly flowing Eastern Australian Current, with juveniles entering estuarine habitats along the New South Wales coast. Ocean Jackets leave estuarine environments before one year of age and mature in nearshore waters at around 35 cm FL and two to three years of age (Miller et al., 2013). Patterns in commercial landings indicate a distinct seasonal pattern, with landings in northern New South Wales almost exclusively coinciding with the winter/spring spawning aggregations, landings through central New South Wales being relatively consistent throughout the year, and landings from southern New South Wales very low during the spawning period and peaking during late summer and autumn. This pattern supports a model of broad-scale seasonal movements of the mature Ocean Jacket biomass northwards during late autumn/winter to spawn, and southwards during late winter to early autumn presumably to feed. Further

work may be needed to resolve population demographics, as there is evidence that that larger and older Ocean Jackets occur further offshore and in deeper waters off South Australia (Lindholm, 1984, Harvey et al., 2013), a possibility for east-coast Ocean Jackets suggested by Gunton et al. (2023) to explain trends in Sr and Ba concentrations with fish age.

Future research may be required to improve understanding of the life-history and population demographics of the western biological stock of Ocean Jackets. Ground-breaking research by Grove-Jones and Burnell (1991) during the late 1980s revealed important insights into the fishery and biology of Ocean Jackets off South Australia. Tag-recapture data showed a pre-spawning westerly migration during late summer and early autumn, with spawning occurring in offshore waters west of Pearson Island, between mid-April and mid-May. A post-spawning return migration was observed from May. Juveniles were found to be seasonal inhabitants of shallow waters of Spencer Gulf and the Yorke Peninsula. This west-east spawning migration may be similar to the proposed north-south migration for the eastern biological stock. The western extent of the spawning biomass requires further investigation.

Fish sampled from Western Australia ranged between 554 and 734 mm TL, being substantially larger than those sampled anywhere else during the study. These fish are approaching the maximum reported length for Ocean Jackets of 790 mm TL (Kailola at el., 1993). Further investigations are required to investigate whether Ocean Jackets in Western Australia represent the oldest and largest individuals in the western spawning stock.

General conclusions, implications and recommendations

The present study has successfully investigated the stock structure of Silver Trevally and Ocean Jackets nationally. This was achieved through a cohesive collaboration between the fisheries management agencies responsible for management of Silver Trevally and Ocean Jacket stocks and key universities. Using a combination of powerful genomic and otolith-based approaches, the study demonstrated the existence of two similarly distributed biological stocks for both Silver Trevally and Ocean Jackets in Australian waters.

Silver Trevally should be considered as comprising two distinct biological stocks in Australian waters. The western biological stock extends from Western Australia to western Victoria, whereas the eastern biological stock extends from eastern Victoria to northern NSW, including north-eastern Tasmania and Commonwealth managed waters off New South Wales.

Similarly, Ocean Jackets should be considered as comprising two distinct biological stocks in Australian waters. The western biological stock extends from Western Australia along the southern Australian coastline to western Victoria, whereas the eastern biological stock extends from northern New South Wales south to eastern Victorian, Tasmanian and east-coast Commonwealth managed waters.

The exact boundaries separating these western and eastern biological stocks remain unknown. However, the results of the present study showing significant differences in both genomics and otolith characteristics suggest a boundary somewhere around central Victoria. Several stocks of important Australian fish species are considered as being separated by Bass Strait, being a substantial barrier to mixing due to oceanography. Important species such as gemfish (*Rexea solandri*), Blue Warehou (*Seriolella brama*), and small pelagics blue mackerel (*Scomber australasicus*), redbait (*Emmelichthys nitidus*), and jack mackerel (*Trachurus declivis*) are considered as distinct genetic stocks (Colgan and Paxton, 1997; Moore et al., 2017, Bulman et al., 2008, Bessell-Browne et al., 2021). Other species, including Jackass Morwong (*Nemadactylus macropterus*) and Pink Ling (*Genypterus blacodes*) are not genetically distinct west and east of Bass Strait, but are managed as such due to observations around limited mixing (Bessell-Browne et al., 2021). It is, therefore, consistent to suggest the western and eastern biological stocks of Silver Trevally and Ocean Jackets be separated for assessment and management by Bass Strait.

Future iterations of SAFS should assess Silver Trevally by biological stock, being a western biological stock (fished in Western Australian, South Australian, western Victorian waters) and an eastern biological stock (fished in New South Wales, east-coast Commonwealth, eastern Victorian and Tasmanian waters). Silver Trevally did exhibit some evidence of limited mixing of adults that could, following further research, support assessment and management at a finer scale than the entire biological stock; however considerable genetic homogeneity within biological stocks, and without evidence of regional spawning migrations or aggregations, suggests that assessment and management at the biological stock scale is appropriate at this time.

Similarly, future iterations of SAFS should assess Ocean Jackets by biological stock, being a western biological stock (fished in Western Australian, South Australian, Commonwealth Great Australian Bight Trawl, and western Victorian waters) and an eastern biological stock (fished in New South Wales, east-coast Commonwealth and eastern Victorian waters). If Ocean Jackets become more common in Tasmanian waters in future years, we conclude they would likely be an extension of the eastern biological stock.

Further development

Silver Trevally and Ocean Jackets both exhibited variations in otolith characteristics that resulted in high classification rates to some locations, suggesting potential for finer-scale population structuring and/or habitat use within the broader biological stocks identified. More constrained sampling to collect fish of similar sizes/ages across locations, in addition to more detailed work into population dynamics and life-history

parameters (e.g., growth rates, size and age at maturity), may help in determining whether finer-scale population structuring is important for assessment and management.

Sampling to refine the extent of the biological stocks and their boundaries is needed for both species. The southern extent of the eastern biological stock of Ocean Jackets needs further investigation through samples obtained from eastern Victorian and Tasmanian waters. This may become more important if any southerly range shift occurs, and if targeting of Ocean Jackets increases. The northern extent of the eastern biological stock of Silver Trevally remains uncertain, and future samples of Silver Trevally from south-eastern Queensland may assist in this understanding. Similarly for the western biological stocks of both species sampling towards the known limits of their ranges would confirm whether finer-scale structuring in these areas is important. More targeted sampling to identify with greater precision where the western and eastern biological stocks are separated is needed, given our sampling could only identify the boundary to be somewhere between Portland/Port Fairy and Corner Inlet.

Future biological stock status assessments would benefit from cross-jurisdictional collaboration in monitoring, data collection, and stock assessment for both Silver Trevally and Ocean Jackets.

Extension and Adoption

The extension and adoption plan for this project had the following objectives:

(i) To ensure that future SAFS assessments are done at appropriate spatial scales

(ii) To ensure that fisheries scientists and managers have access to new knowledge that could influence monitoring and assessment

(iii) To ensure that key stakeholders and the public are aware of the main research outcomes arising from the project

(iv) To ensure new scientific knowledge, including any advances in the analytic methods undertaken, are communicated to the wider scientific community.

These will be achieved through publication of the final report and dissemination to the SAFS advisory group, and relevant managers and scientists across all agencies responsible for management of Silver Trevally and Ocean Jackets. Presentations will be provided for relevant Resource Advisory Groups including the South East Resource Assessment Group (SERAG) and the Great Australian Bight Resource Assessment Group (GABRAG). Results will be published through scientific papers in international journals.

During the project regular project team meetings were held via Microsoft TEAMS to share progress and discuss issues. All fishers who contributed to the project in terms of samples were contacted and thanked, with most also being sent a one-page summary document detailing the stock structures and boundaries. An article detailing the project, and its findings was published in the FRDC online newsletter in August 2024.

Appendices

Silver Trevally otoliths supplementary material

Otolith Shape

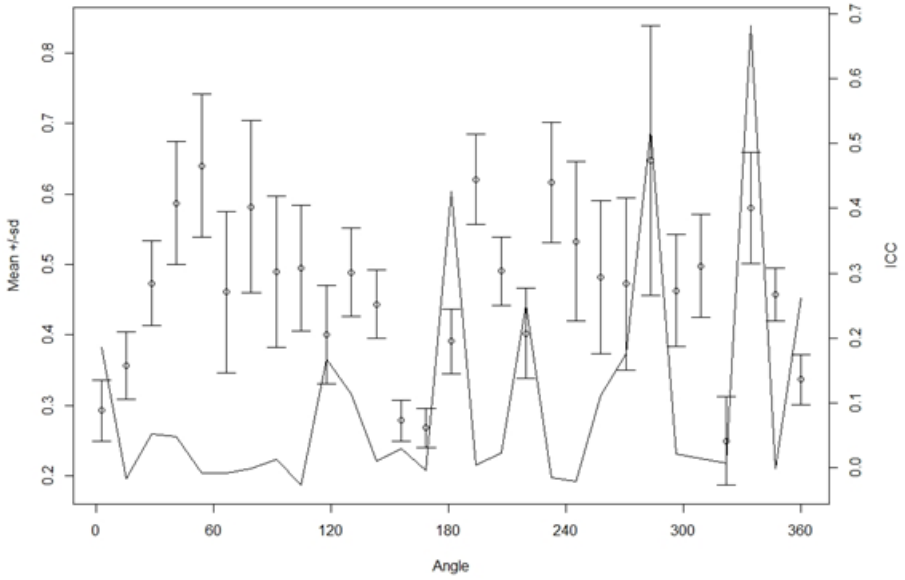

Figure S1. Mean and standard deviation (sd) of the Wavelet coefficients for all Silver Trevally otoliths. Also shown is the proportion of variance among groups represented by intraclass correlation (ICC, black solid line). The horizontal axis shows angle in degrees (°) based on polar coordinates.

Table S1. Summary of correct classifications (%) of Silver Trevally to individual locations and regions (pooling locations within and among jurisdictions and broader regions) based on canonical analysis of principle coordinates (CAP) and otolith shape. Each column shows results for different models (a-e) and the classification accuracy per location or pool of broader regions, as well as the overall correct classification accuracy (overall %). Models go from a) including all locations to e) pooling locations to compare an East – West grouping with a boundary within Victoria. Also shown is the number of individuals per location (N inds).

Jurisdiction	**Location**	**N inds**	**Correct classification (%)**				
			a)	**b)**	**c)**	**d)**	**e)**
New South Wales (Commonwealth)	Bermagui	30	10	34	60	90	90
	Sydney	30	13				
	Ulladulla	30	13				
New South Wales	Bermagui	15	33	51			
	Coffs Harbour	30	20				
	Jervis Bay	30	17				
	Sydney	29	21				
Tasmania	North Tasmania	30	13	47	53		
Victoria	Corner Inlet	30	60	47	47		
	Port Fairy	15	40				84
South Australia	Neptune Islands	28	32	23	29	79	
	Venus Bay	20	0				
Western Australia	Metropolitan (Perth)	17	35	53	55		
	South Coast East(Esperance)	23	17				
	South Coast West (Albany)	30	13				
	South-West (Dunsborough)	30	7				
Total N / Overall %		**417**	**21%**	**43%**	**53%**	**83%**	**86%**

Otolith chemistry

Table S2. Summary of correct classifications (%) of Silver Trevally to individual locations and regions (pooling locations within and among jurisdictions and broader regions) based on canonical analysis of principle coordinates (CAP) and otolith chemical composition. Each column shows results for different models (a-e) and the classification accuracy per location or pool of broader regions, as well as the overall correct classification accuracy (overall %). Models go from a) including all locations to e) pooling locations to compare an East – West grouping with a boundary within Victoria. Also shown is the number of individuals per location (N inds).

Jurisdiction	**Location**	**N inds**	**Correct classification (%)**				
			a)	**b)**	**c)**	**d)**	**e)**
New South Wales (Commonwealth)	Bermagui	30	17	13	48	69	79
	Sydney	38	37	45			
	Ulladulla	29	7	24			
New South Wales	Bermagui	22	41	41	59		
	Coffs Harbour	39	84	85			
	Jervis Bay	39	77	46			
	Shoalhaven Coast	22	4				
	Shoalhaven River	19	47				
	Sydney	39	67	64			
Tasmania	Low Head	29	34	54	60	63	
	North Tasmania	39	56				
Victoria	Corner Inlet	36	67	80	80		
	Port Fairy	39	51	54		64	73
South Australia	Cape Elizabeth	13	15	23	44		
	Neptune Islands	30	30	37			
	Venus Bay	30	43	40			
Western Australia	Metropolitan (Perth)	34	27	36	64	69	
	Mid West (Geraldton)	21	48	52			
	South Coast East(Esperance)	32	19	9			
	South Coast West (Albany)	34	41	44			
	South-West (Dunsborough)	22	23	27			
Total N / Overall %		**656**	**42%**	**46%**	**59%**	**67%**	**76%**

Ocean Jacket otoliths supplementary material

Otolith Shape

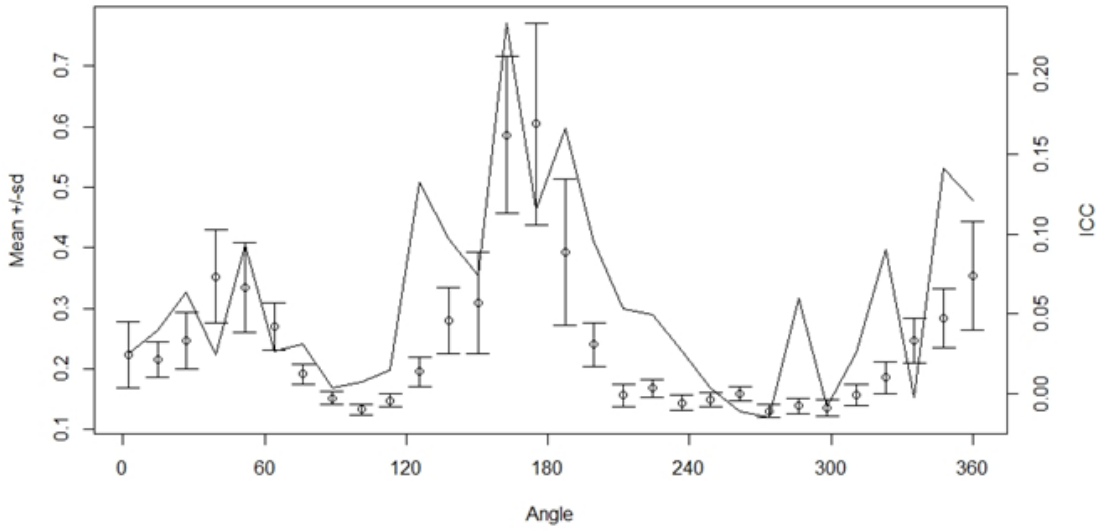

Figure S2. Mean and standard deviation (sd) of the Wavelet coefficients for Ocean Jacket otoliths. Also shown is the proportion of variance among groups represented by intraclass correlation (ICC, black solid line). The horizontal axis shows angle in degrees (°) based on polar coordinates.

Table S3. Summary of correct classifications (%) of Ocean Jackets to individual locations and regions (pooling locations within and among jurisdictions and broader regions) based on canonical analysis of principle coordinates (CAP) and otolith shape. Each column shows results for different models (a-c) and the classification accuracy per location or pool of broader regions, as well as the overall correct classification accuracy (overall %). Models go from a) including all locations to c) pooling locations to compare an East – West grouping with a boundary between Victoria and South Australia. Also shown is the number of individuals per location (N inds).

Jurisdiction	**Location**	**N inds**	**Correct classification (%)**		
			a)	**b)**	**c)**
New South Wales (Commonwealth)	Greencape	16	13	0	54
	Ulladulla	31	41		
New South Wales	Coffs Harbour	26	12	100	
	Jervis Bay	27	7		
	Narooma	36	8		
	Terrigal	25	0		
Victoria	Portland	27	0	0	
South Australia	Coffin Bay	23	0	0	57
	Port Lincoln	32	31		
Western Australia	South-West (Augusta)	25	4	0	
	South-West (Hamelin)	23	13		
Total N / Overall %		**289**	**13%**	**37%**	**55%**

Table S4. Summary of correct classifications (%) of Ocean Jackets to individual locations and regions (pooling locations within and among jurisdictions and broader regions) based on canonical analysis of principle coordinates (CAP) and otolith chemical composition. Each column shows results for different models (a-d) and the classification accuracy per location or pool of broader regions, as well as the overall correct classification accuracy (overall %). Models go from a) including all locations to d) pooling locations to compare an East – West grouping with a boundary between Victoria and South Australia. Also shown is the number of individuals per location (N inds).

Jurisdiction	**Location**	**N inds**	**Correct classification (%)**			
			a)	**b)**	**c)**	**d)**
New South Wales (Commonwealth)	Eden	20	70	80	72	76
New South Wales	Coffs Harbour	27	67	68		
	Jervis Bay	28	57			
	Narooma	29	79			
	Terrigal	27	82			
Victoria	Portland	28	64	64	68	
South Australia	Coffin Bay	31	0	37	45	74
	Port Lincoln	29	35			
Western Australia	South-West (Augusta)	27	59	59	52	
Total N / Overall %		**246**	**56%**	**60%**	**63%**	**75%**

Researchers and project staff

Dr John Stewart, scientist, NSW Department of Primary Industries and Regional Development

Ms Julie Rehwinkel, business partner, NSW Department of Primary Industries and Regional Development

Dr Ash Fowler, scientist, NSW Department of Primary Industries and Regional Development

Dr Tom Barnes, scientist, NSW Department of Primary Industries and Regional Development

Dr Rowan Chick, scientist, NSW Department of Primary Industries and Regional Development

Dr Amy Smoothey, scientist, NSW Department of Primary Industries and Regional Development

Dr Julian Hughes, scientist, NSW Department of Primary Industries and Regional Development

Ms Chantelle Clain, technician, NSW Department of Primary Industries and Regional Development

Mr Nick Meadows, technician, NSW Department of Primary Industries and Regional Development

Professor Luciano Beheregaray, Flinders University

Associate Professor Luciana Moller, Flinders University

Professor Charlie Huveneers, Flinders University

Ms Zoriana Lam, Flinders University

Professor Bronwyn M Gillanders, The University of Adelaide

Dr Patrick Reis-Santos, The University of Adelaide

Ms Rhiannon Van Eck, The University of Adelaide

Dr Corey Green, scientist, Victorian Fisheries Authority

Mr Scott Gray, contractor biological sampling

Dr Sean Tracey, scientist, University of Tasmania

Dr Nils Krueck, scientist, University of Tasmania

Dr Michael Drew, scientist, South Australian Research and Development Institute

Dr David Fairclough, scientist, Department of Primary Industries and Regional Development

Dan Corrie, manager, Australian Fisheries Management Authority (AFMA)

References

Abdi, H. and Williams, L.J. 2010. Principal component analysis. Wiley interdisciplinary reviews: computational statistics, 2(4), pp.433-459.

Alexander, D.H., Novembre, J. and Lange, K. 2009. Fast model-based estimation of ancestry in unrelated individuals. Genome research, 19(9), pp.1655-1664.

Annala, J.H., Sullivan, K.J. and O'Brien, C.J. (compilers). 1999. Report from the Fishery Assessment Plenary, April 1999: stock assessments and yield estimates. 430 pp Unpublished report held in NIWA Library, Wellington. MAF, New Zealand.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral ecology, 26(1), pp.32-46.

Anderson, M.J. and Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology, 84(2), pp.511-525.

Bearham, D., Robert, M., Chaplin, J.A., Moore, G.I., Fairclough, D.V. and Bertram, A. 2020. Molecular evidence of three species in the *Pseudocaranx dentex* complex (Carangidae) in Australian waters. Marine and Freshwater Research, 71(4):518-31.

Benjamini, Y. and Yekutieli, D. 2001. The control of the false discovery rate in multiple testing under dependency. Annals of statistics, pp.1165-1188.

Bessell-Browne, P., Day, J., Sporcic, M. and Appleyard, S., 2021. SESSF species stock structure review: Blue Warehou, Jackass Morwong and Pink Ling. Australian Fisheries.

Bulman, C., Condie, S., Findlay, J., Ward, B. and Young, J., 2008. Management zones from small pelagic fish species stock structure in southern Australian waters. FRDC project, (2006/076).

Burch, P., Fowler, A., Liggins, G., Sporcic, M., Dowling, N., Chick, R., Tuck, G. 2023. A joint Commonwealth and NSW assessment of Silver Trevally (*Pseudocaranx georgianus*). Prepared for the Australian Fisheries Management Authority. 83 pp.

Colgan, D.J. and Paxton, J.R., 1997. Biochemical genetics and recognition of a western stock of the common gemfish, *Rexea solandri* (Scombroidea: Gempylidae), in Australia. Marine and Freshwater Research, 48(2), pp.103-118.

Dixon, P.I. and Musa, J. 1995. Genetic Structure of Ocean Jacket Populations – Pilot Study. Final Report, FIRDC 91/26). Centre for Marine Science at the University of New South Wales.

Fairclough, D.V., Potter, I.C., Lek, E., Bivoltsis, A.K. and Babcock, R.C. 2011. The fish communities and main fish populations of the Jurien Bay Marine Park. Centre for Fish and Fisheries Research.

Farmer, B.M., French, D.J.W., Potter, I.C., Hesp, S.A. and Hall, N.G. 2005. Determination of biological parameters for managing the fisheries for Mulloway and Silver Trevally in Western Australia. Centre for Fish and Fisheries Research, Murdoch University.

Fowler, A.M., Chick, R.C. and Stewart, J. 2018. Patterns and drivers of movement for a coastal benthopelagic fish, *Pseudocaranx georgianus*, on Australia's southeast coast. Scientific Reports, 8(1), 16738.

Fowler, A., Chick, R.C., Krueck, N., Fairclough, D., Victorian Fisheries Authority, Emery, T., Roelofs, A., Rogers, P., and Fisher, E. Silver Trevallies (*Pseudocaranx georgianus*, *Pseudocaranx sp*. "*dentex*" & *Pseudocaranx wrighti*, *Pseudocaranx dinjerra*), in Piddocke, T., Ashby, C., Hartmann, K., Hesp, A., Klemke, J., Krueck, N., Mayfield, S., Penny, S., Roelofs, A., Saunders, T., Soe Hlaing, K., Stewart, J., Wise, B. and

Woodhams, J. (eds) 2021. Status of Australian fish stocks reports 2020, Fisheries Research and Development Corporation, Canberra. https://fish.gov.au/

Grove-Jones R.P. and Burnell A.F. 1991. Fisheries biology of the ocean leatherjacket (Monacanthidae: *Nelusetta ayraudi*) in the eastern waters of the Great Australian Bight, South Australia. Fishing Industry Research and Development Council, Grant No: DFS01Z, pp 1–107

Gruber, B., Unmack, P. J., Berry, O. F., and Georges, A. 2018. dartr: An r package to facilitate analysis of SNP data generated from reduced representation genome sequencing. Molecular Ecology Resources, 18(3), 691–699. https://doi.org/10.1111/1755-0998.12745

Gunton, H., Fowler, A.M., Miller, M.E., Booth, D.J. and Stewart, J., 2023. The utility of otolith-based methods for discriminating stock structure of the fishery important monacanthid, *Nelusetta ayraud*, over an intermediate scale. Fisheries Research, 264, p.106715.

Harvey, E.S., Cappo, M., Kendrick, G.A., Mclean, D.L. 2013. Coastal fish assemblages reflect geological oceanographic gradients within an Australian zootone. PloS One 8 (11), e80955.

Hutchins, B. 2000. Leatherjackets. In: Andrew N (ed) Under Southern Seas: the ecology of Australia's rocky reefs. Krieger, Malabar, pp 194–201

Izzo, C., Ward, T.M., Ivey, A.R., Suthers, I.M., Stewart, J., Sexton, S.C. and Gillanders, B.M. 2017. Integrated approach to determining stock structure: implications for fisheries management of sardine, *Sardinops sagax*, in Australian waters. Reviews in fish biology and fisheries, 27, pp.267-284.

Jaccoud, D., Peng, K., Feinstein, D., and Kilian, A. 2001. Diversity arrays: a solid state technology for sequence information independent genotyping. Nucleic Acids Research, 29(4), e25. https://doi.org/10.1093/nar/29.4.e25

Jackson, G., Fairclough D.V., Fisher, E.A., Hetzel, Y., Van Der Mheen, M., Scoulding, B., Gillanders, B.M., Reis-Santos, P., Beheregaray, L.B., Sandoval-Castillo, J. and Braccini M. 2023. Where did the Snapper go? Determining factors influencing the recovery of Snapper (Chrysophrys auratus) stocks on the West coast of Australia. Final Report on FRDC Project No. 2018-050. Perth, Western Australia

James, G.D. 1980. Tagging experiments on trawl-caught trevally, *Caranx georgianus*, off north-east New Zealand, 1973–79. New Zealand Journal of Marine and Freshwater Research, 14(3), pp.249-254.

Jolliffe, I.T. 2002. Principal component analysis for special types of data (pp. 338-372). Springer New York.

Jombart, T. and Ahmed, I. 2011. adegenet 1.3-1: new tools for the analysis of genome-wide SNP data. Bioinformatics, 27(21), pp.3070-3071.

Kailola P.J., Williams M.J., Stewart P.C., Reichelt R.E., McNee A. and Grieve C. 1993, Australian Fisheries Resources. Australian Bureau of Resource Sciences and the Fisheries Research and Development Corporation. Canberra.

Kassambara, A. and Mundt, F. 2020. Factoextra: Extract and visualize the results of multivariate data analyses. v1. 0.7. CRAN. R-project. org/package= factoextra.

Kemp, L. 2020. A first investigation into the evolutionary relationships, population structure and demographic history of New Zealand Trevally (*Pseudocaranx georgianus*) and its implications for fisheries management. Open Access Te Herenga Waka-Victoria University of Wellington. Thesis. https://doi.org/10.26686/wgtn.17147750

Libungan L.A., Pálsson S. 2015. ShapeR: An R Package to Study Otolith Shape Variation among Fish Populations. PLOS ONE 10(3): e0121102

Lindholm, R., 1984. Observations on the chinaman leatherjacket *Nelusetta ayraudi* (Quoy and Gaimard) in the Great Australian Bight. Aust. J. Mar. Freshw. Res. 35, 597–599.

Mantel, N. 1967. Ranking procedures for arbitrarily restricted observation. Biometrics, pp.65-78.

Mijangos, J.L., Gruber, B., Berry, O., Pacioni, C. and Georges, A. 2022. dartR v2: An accessible genetic analysis platform for conservation, ecology and agriculture. Methods in Ecology and Evolution, 13(10), pp.2150-2158.

Miller, M.E. and Stewart, J., 2009. The commercial fishery for ocean leatherjackets (*Nelusetta ayraudi*, Monacanthidae) in New South Wales, Australia. Asian Fish Sci, 22, pp.257-264.

Miller, M.E., Stewart, J., West, R.J. 2010. Using otoliths to estimate age and growth of a large Australian endemic monocanthid, *Nelusetta ayraudi* (Quoy and Gaimard, 1824). Environ. Biol. Fishes 88 (3), 263–271.

Miller, M.E. and Stewart, J. 2013. Reproductive characteristics of the ocean leatherjacket, *Nelusetta ayraudi*. Reviews in fish biology and fisheries, 23(1), pp.87-101.

Moore, A., Ovenden, J. and Bustamante, C. 2017. Research to underpin better understanding and management of western Gemfish stocks in the Great Australian Bight. FRDC Project 2014/104. Australian Bureau of Agricultural and resource Economics and Sciences, Canberra, September 2017.

Neira, F.J. and Miskiewicz, A.G. 1998. Larvae of temperate Australian fishes: laboratory guide for larval fish identification. UWA Publishing.

Novembre, J. and Stephens, M. 2008. Interpreting principal component analyses of spatial population genetic variation. Nature genetics, 40(5), pp.646-649.

Paton, C., Hellstrom, J., Paul, B., Woodhead, J., Hergt, J. 2011. Iolite: Freeware for the visualisation and processing of mass spectrometric data. Journal of Analytical Atomic Spectrometry 26, 2508-2518.

R Core Team. 2023. R: A language and environment for statistical Computing. R Foundation for Statistical Computing.

Reis-Santos, P., Vasconcelos, R.P., Tanner, S.E., Fonseca, V.F., Cabral, H.N. and Gillanders, B.M. 2018. Extrinsic and intrinsic factors shape the ability of using otolith chemistry to characterize estuarine environmental histories. Marine Environmental Research, 140, pp.332-341.

Rowling, K.R, Raines, L.P. 2000. Description of the biology and an assessment of the fishery of Silver Trevally *Pseudocaranx dentex* off New South Wales. Final Report to Fisheries Research and Development Corporation. Project no. 97/125.

Smith-Vaniz, W.F. and Jelks, H.L. 2006. Australian trevallies of the genus Pseudocaranx (Teleostei: Carangidae), with description of a new species from Western Australia. Memoirs of Museum Victoria, 63(1), pp.97-106.

Stransky, C. 2014. Chapter Seven - Morphometric Outlines. In: Cadrin S.X., Kerr, L.A., Mariani, S. (eds) Stock Identification Methods (Second Edition). Academic Press, San Diego.

Sunnucks, P. and Hales, D.F. 1996. Numerous transposed sequences of mitochondrial cytochrome oxidase I-II in aphids of the genus Sitobion (Hemiptera: Aphididae). Molecular biology and evolution, 13(3), pp.510-524.

Venables, W. N. and Ripley, B. D. 2002. Modern Applied Statistics with S. Fourth edition. Springer.

Wickham, H. and Bryan, J. 2019. readxl: Read Excel Files. R package version 1.3. 1.

Wickham, H. 2016. Getting Started with ggplot2. ggplot2: Elegant graphics for data analysis, pp.11-31.

Yoshinaga, J., Nakama, A., Morita, M., Edmonds, J.S. 2000. Fish otolith reference material for quality assurance of chemical analyses. Marine Chemistry 69, 91-97.