

# Understanding the stock structure of Rock Flathead and the role of movement dynamics in influencing the performance of the Corner Inlet Fishery



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### Abbreviations

AMOVA - Analysis of molecular variance BP - base pair CAP - Canonical analysis of principal coordinates DNA - Deoxyribonucleic acid FST - Fixation index LAICP-MS - Laser ablation inductively coupled plasma-mass spectrometry LD - Linkage disequilibrium LOO - Leave-one-out cross validation MAF - Minor allele frequency PCA - Principal components analysis PCR - Polymerase chain reaction PERMANOVA - Permutational multivariate analysis of variance SNP - Single nucleotide polymorphism VFA - Victorian Fisheries Authority

### **Executive Summary**

Rock Flathead (*Platycephalus laevigatus*) is a marine fish that inhabits shallow seagrass habitats across southern Australia, with a distribution extending from Greenwell Point in New South Wales to Geographe Bay in Western Australia, including Tasmania. The species supports recreational fisheries across its range, and a single commercial fishery in Corner Inlet in eastern Victoria. Commercial catches of Rock Flathead from Corner Inlet suffered a significant decline between the mid and late 1990s, with commercial catches dropping from 92 tonnes to 30 tonnes over a 5-year period (Koopmen et al. 2004). The exact driver(s) of this decline remains uncertain but highlights the need for improved knowledge on the resilience of local fishing stocks to fishing pressure and environmental disturbance to inform the sustainable management of the fishery into the future. At present, information on the biological connections between Rock Flathead fishing stocks across south-eastern Australia is currently limited, creating uncertainty around whether the Corner Inlet Fishery is an isolated, self-replenishing fishing stock, requiring independent management consideration. This has been recognised as a major limitation for managers responsible for assessing the status of the fishery and in ensuring its long-term sustainability.

This project involves a direct collaboration between Deakin University, the Victorian Fisheries Authority, industry stakeholders and the Fisheries Research and Development Corporation. The aim of the project was to adopt an integrated approach, involving population genomics, otolith microchemistry, and acoustic telemetry, to address key questions regarding patterns of biological connectivity among Rock Flathead fishing stocks, with a specific focus on the fishery at Corner Inlet. Specific questions addressed include:

- 1. Is the Corner Inlet Rock Flathead population an isolated, self-replenishing stock or not?
- 2. If not, what is the broader population structure and connectivity of Rock Flathead in south-east Australia, and how does the Corner Inlet population relate to other populations?
- 3. How do movement behaviours, both within Corner Inlet and between Corner Inlet and coastal waters, influence population and fishery dynamics?

We found consistent results across all survey methods, pointing to limited fish movement between Corner Inlet and stocks outside the inlet. Specifically, analyses of population genetic structure indicated significant genetic structuring and gene flow limitations across the sampling distribution that included Victorian and Tasmanian embayment and open coastal habitats, indicating the Corner Inlet Fishery to be an isolated gene pool and genetically distinct from all other stocks. Similarly, analyses of otolith microchemistry suggested chemical profiles of fish from Corner Inlet to be distinct from all other sample locations from south-eastern Australia, indicating restricted dispersal across both juvenile and adult life stages. Finally, acoustic telemetry indicated individual fish movements to be highly localised, with movements between Corner Inlet fish and other locations being unlikely. Although data obtained from acoustic telemetry was limited due to detections from limited individuals and unexpected loss of receivers, our weighted evidence approach suggests that the Corner Inlet Rock Flathead fishery is an isolated, self-recruiting stock, which requires independent management consideration. We discuss the management implications of these findings, specifically in relation to the assessment of fisheries resilience to commercial fishing pressure and environmental disturbance. This new information will provide a much-needed resource to assist managers in future stock assessments and sustainable management of the Corner Inlet Rock Flathead fishery.

#### **Keywords**

Rock Flathead, biological stock connectivity, south-eastern Australia, population genomics, otolith microchemistry, acoustic telemetry, sustainable management.

### Introduction

The Rock Flathead, Platycephalus laevigatus, is a long lived, slow growing demersal marine fish species that inhabits rocky reef, sand and seagrass habitats in shallow, inshore waters of southern Australia. The species has a wide distribution extending from Greenwell Point (New South Wales) to Geographe Bay (Western Australia), including Tasmania, with the only commercial fishery operating in Corner Inlet in eastern Victoria, and relatively small recreation catches occurring across the species range, including Corner Inlet, Port Phillip Bay, and Western Port in Victoria (Koopman et al. 2004; Kemp et al. 2014). Historically, Rock Flathead has supported a commercial mesh-net and haul seine fishery in Corner Inlet Fishery, however the fishery suffered significant declines between 1993 to 1998, with commercial catches reduced by 67% from 92 t to 30 t (Koopman et al. 2004). While the reason for these declines remains largely uncertain, evidence suggests these might be linked to the increase in mesh net fishing effort during the early 1990s (Koopman et al. 2004). Since then, there have been positive signs of stock recovery with catches increasing to 69 t in the 2022/23 season (Bell et al. 2024) and the most recent stock assessment indicating the fishery to be in a good condition, with a ten-year average catch rate of 0.71 kg/km-hour and stocks determined to be above a 30-year average (Kemp et al. 2014). However, commercial landings remain lower than historical averages (prior to the decline in the mid 1990's) and the industry has recognised the importance of addressing critical knowledge gaps associated with the stock status to sustainably manage this valuable fishery into the future.

The Victorian Fisheries Authority (VFA) recently released a management plan for Corner Inlet fisheries, including Rock Flathead, identifying key targets and management approaches for both commercial and recreational fisheries, to ensure the sustainability of local fisheries into the future (Victorian Fisheries Management Authority 2022). At present, commercial fisheries have limited entry and a maximum of 18 single operator licenses, with gear restrictions (i.e., net length and mesh size limits and compulsory catch and effort reporting. Similarly, the recreational is managed using a combination of various input controls (e.g., gear restrictions) and species' size, bag and possession limits, with all fishing (i.e., both commercial and recreational) being prohibited within the Corner Inlet Marine National Park. The Corner Inlet Fisheries Management Plan also recognises the need for investment in key research areas to help overcomes knowledge gaps and to mitigate perceived risks to local fisheries. The VFA and local stakeholders recognise the risk that commercial harvest of Rock Flathead from Corner Inlet could lead to recruitment overfishing, with a key management response being to better understand Rock Flathead stock structure and movement dynamics. Specifically, information is needed to determine if the Corner Inlet Rock Flathead fishery is an isolated, selfrecruiting fishing stock.

Sustainable fisheries management hinges on an understanding of biological stock structure, including information on the geographic boundaries of fish populations and the natural recruitment potential of individual stocks persisting within and across these populations (Papa et al. 2021). Furthermore, understanding patterns of biological connectivity among fishing stocks is also fundamental for identifying key habitats for protection, appreciating the spatial reach of disturbance events (including the spread of disease), and when interventions might be needed to help recover depleted stocks (i.e., restocking; Bell et al. 2006). In general, spatial patterns of biological connectivity between Rock Flathead fishing stocks across southern Australia remain uncertain but are needed to effectively gauge the resilience of stocks to commercial and recreational fishing pressure and environmental disturbance. Evidence from a sister species, Dusky Flathead (Platycephalus fuscus), suggests a high level of connectivity among stocks spanning the entire New South Wales coastline and the recognition of a single admixed stock based on genetic data (Taylor et al. 2020). Anecdotally, commercial fishers believe that Rock Flathead are highly mobile and freely move in and out of Corner Inlet into the open ocean, particularly during spawning seasons. Whilst such movement patterns could potentially contribute to the admixture of stocks at regional scales, empirical data on animal movement and stock connectivity remains limited in Rock Flathead.

Multiple tools are used widely for estimating biological connections among natural populations of marine species supporting commercial fisheries. Population genetic tools are commonly used to help delineate stock boundaries through direct assessments of spatial patterns of gene exchange among fishing stocks (Bertram et al. 2022; Miller et al. 2016). The field of population genetics has benefited greatly from modern developments in DNA sequencing technologies that allow for genome-wide assessments of genetic variation and provide unprecedented sensitivity for resolving fine scale signals of population genetic structure (Cheng et al. 2021; Miller et al. 2019). Further insights can be gained from the analysis of otolith microchemistry profiles which reflect patterns of animal movement and habitat use (Campana 1999). Specifically, chemical elements from the local environment are deposited into the calcium carbonate otolith structures, producing chemical signatures in discrete layers that are reflective of the local environment occupied by the fish at the time of deposition (Campana and Thorrold 2001). Finally, satellite and acoustic telemetry approaches are widely used for charactering animal movement patterns and have been pivotal in understanding species spatial ecologies including dispersal extent, habitat use and population connectivity (Crossin et al. 2017). Despite the opportunities provided by each of these methods,

each has its own limitations. For example, true extent of connectivity between stocks, can be difficult to quantify in the absence of significant genetic structure (Fish et al. 2024). Homogeneity in otolith microchemistry profiles can occur in the absence of significant chemical gradients between local environments where stocks are being contrasted (Sturrock et al. 2012). Furthermore, telemetry studies are often poorly replicated due to cost and the spatial extent of acoustic listening stations (Hellström et al. 2016). Consequently, the most reliable insights into the biological connections between commercial fishing stocks are gained when these tools are used in combination.

In this study we use a multi-disciplinary approach to investigate patterns of biological connectivity among Rock Flathead populations from south-eastern Australia, with a particular focus on the status of the of the Corner Inlet Fishery. The project involved a direct collaboration between Deakin University, the Victorian Fisheries Authority, industry stakeholders and the Fisheries Research and Development Corporation. First, we used a population genomic approach (assessment of genome wide genetic variation) to investigate patterns of gene flow and genetic structure among Rock Flathead from Corner Inlet, Port Phillip Bay, Flinders Island, and north-western Tasmania. Next, we contrasted otolith microchemistry profiles from fish sampled from each of these locations to gain further insights into patterns of animal movement and habitat usage across life stages. Finally, we used acoustic telemetry to track the movement patterns of individual Rock Flathead within and outside of Corner Inlet using an array of acoustic receivers. Using these approaches, we set out to address the following questions:

- 1. Is the Corner Inlet Rock Flathead population an isolated resident, self-replenishing stock?
- 2. What is the broader population structure and connectivity of Rock Flathead in south-east Australia, and how does the Corner Inlet population relate to other populations?
- 3. How do movement behaviours, both within Corner Inlet and between Corner Inlet and coastal waters, influence population and fishery dynamics?

Outputs from this research project provide new insights into patterns of biological connectivity in Rock Flathead and challenges current assumptions of stock admixture across the fishery. We discuss the management implications of these findings, specifically in relation to the assessment of fisheries resilience to commercial fishing pressure and environmental disturbance. We expect this new information will provide a much-needed resource for assisting managers in future stock assessments and sustainable management of Corner Inlets valuable Rock Flathead fishery.

# **Objectives**

The objective of this study is to:

- 1. Determine the population structure of Rock Flathead in south-eastern Australia, with emphasis on understanding how the Corner Inlet population relates to other populations;
- 2. Characterise the movement and residency patterns of Rock Flathead in Corner Inlet.

## Methods

#### **Collection of biological samples**

A total of 297 Rock Flathead frames were collected from Corner Inlet (CI; n=184) and Port Phillip Bay (PPB; n=51) in Victoria, north-western Tasmania (M, RC, S, W; n=54) and Flinders Island (FI; n=8) in Bass Strait between 9 April 2021 and 25 January 2022 (Figure 1; Table 1). All fish frames were collected by commercial fishermen (CI, PPB, TAS, FI) or obtained from seafood retail outlets (CI) and were frozen prior to processing for genetic and microchemistry analysis. Frames were subsequently processed at the University of Tasmania (Launceston campus) and Deakin University (Queenscliff campus), where sagittal otolith pairs were removed using ceramic forceps from all individual fish frames, gently wiped dry and stored in labelled envelopes for downstream microchemistry analysis. Muscle tissue biopsies (approximately 2 g) were extracted from up to 33 individual frames per location for population genomic analysis. Biopsies were taken using scalpels and forceps that were sterilised between samples to avoid cross-contamination and preserved individually in 1.5 ml microcentrifuge tubes containing 100% ethanol until required for genetic analysis.



**Figure 1.** Map showing locations where Rock Flathead samples were collected for population genomic and otolith microchemistry analyses.

**Table 1.** Details of Rock Flathead sample locations and total numbers of samples from each location

 included in the population genomic and otolith microchemistry analyses.

| Region         | Location         | Code | Latitude   | Longitude   | No. of<br>samples<br>(genomics) | No. of<br>samples<br>(microchem) |
|----------------|------------------|------|------------|-------------|---------------------------------|----------------------------------|
| Region         | Location         | couc | Lutituuc   | Longitude   | (genonics)                      | (interochenny                    |
| Victoria       | Corner Inlet     | CI   | -38.768445 | 146.336259  | 33                              | 186                              |
|                | Port Phillip Bay | PPB  | -38.216241 | 144.854154  | 33                              | 53                               |
| Bass<br>Strait | Flinders Island  | FI   | -40.321775 | 148.161934  | 8                               | 8                                |
| Tasmania       | Montagu          | Μ    | -40.73351  | 144.9960000 | 5                               | 54*                              |
|                | Rocky Cape       | RC   | -40.85616  | 145.524325  | 9                               |                                  |
|                | Stanley          | S    | -40.756553 | 145.309557  | 33                              |                                  |
|                | Wynard           | W    | -40.974491 | 145.747829  | 4                               |                                  |

\* Samples from Tasmanian locations were pooled for microchemistry analysis due to small sample sizes at individual locations and evidence of a lack of stock structure from the population genomic analysis (described below).

#### **Population genomics**

Total genomic DNA was extracted from 10-15mg of muscle tissue for each of the 125 tissue biopsies by Diversity Arrays Technologies (DArT Pty Ltd, Canberra, Australia) using a NucleoMag 96 tissue kit (Macherey- Nagel, Düren, Germany) coupled with NucleoMag SEP (Ref. 744900) to allow automated separation of high-quality DNA on a Freedom Evo robotic liquid handler (TECAN, Männedorf, Switzerland). Single Nucleotide Polymorphism (SNP) genotyping was performed using the genomewide and high-density DArTseq<sup>™</sup> platform specifically developed for Rock Flathead (*P. laevigatus*) (Sansaloni et al. 2011). This involved a combination of genome complexity reduction methods, including a DNA digestion and ligation step using PstI/Taql restriction enzymes, followed by PCR and quantification (Sansaloni et al. 2011; Kilian et al. 2012). Samples are then standardised, and pooled for sequencing using an Illumina HiSeq2500 instrument, with DNA sequence data filtered and assembled using the DArTseq<sup>™</sup> analytical pipeline. The DArTseq<sup>™</sup> algorithm uses technical replicates to calculate genotyping reproducibility and Mendelian inheritance patterns to filter paralogous regions and sequencing errors (Sansaloni et al. 2011; Kilian et al. 2012).

Genotyping with the DArTseq<sup>™</sup> platform yielded a total of 8,765 SNP markers. To ensure only the highest quality markers were used in downstream population genomic analyses, we implemented a series of stringent SNP filtering steps. First, we excluded SNP loci with a high proportion of missing data by using a call rate threshold of 90% for both individual loci and individual samples. To avoid erroneous SNP genotypes due to sequencing error we enforced a minimum read depth of 10 and controlled for potential linkage disequilibrium between loci using a hamming distance threshold of 0.2. Finally, SNPs were called using a minor allele frequency (MAF) setting of 0.02. All the filtering steps were performed using the *DaRTR* package (Gruber et al. 2018) implemented in R (R Core Team, 2019). Filtering resulted in a final data set containing 236 high quality SNP loci that were subsequently used for downstream tests for population genetic differentiation.

Descriptive statistics were calculated for the SNP data using the *HierFstat* package (Goudet 2004) implemented within R, including: (a) allelic richness per population averaged over loci; (b) observed and expected heterozygosities; (c) Weir and Cockerham's inbreeding coefficient ( $F_{IS}$ ) and global population differentiation ( $F_{ST}$ ) with 95% confidence limits (Weir & Cockerham, 1984); and (d) population pairwise measures of  $F_{ST}$  with significance determined using permutation (10,000). An

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analysis of molecular variance (AMOVA) was performed in GenAlex (Peakall and Smouse 2006) partitioning genetic variation among regions (Victoria vs Flinders Island vs Tasmania), sample site within regions, and among individuals within sites. Discriminant analysis of principle components (DAPC) using the 'find clusters' function and principal components analysis (PCA), both implemented in the R package *Adegenet* (Jombart 2008), were used to summarize patterns of genetic differentiation between sample locations. Finally, Bayesian analyses were conducted to estimate the number of populations within the sample data using the software package STRUCTURE (Pritchard et al. 2000) which identifies the number of distinct population clusters, assigns individuals to clusters, and identifies migrants and admixed individuals using genetic data only. To determine the number of populations (*K*), 10 independent simulations for *K* = 1–7 with 25,000 burn-in and 500,000 data iterations were run. Analyses were performed using the admixture model of population structure (i.e. each individual draws some fraction of their genome from each of *K* populations) and allele frequencies were set as independent among populations. The most likely *K* was estimated using Evanno's  $\Delta K$  (Evanno et al. 2005) in Structure Harvester (Earl & vonHoldt 2012).

#### **Otolith microchemistry**

A single otolith per sample was prepared for trace element spot analysis using laser ablation inductively coupled plasma-mass spectrometry (LAICP-MS) following the methods described in Gillanders (2002). Sectioned otoliths were mounted onto a total of six glass slides using indium spiked Crystalbond 509 temporary adhesive prior to the analytical process. Indium was used as an internal elemental marker of the Crystalbond 509 adhesive and epoxy. Analyses were performed using the LA ICP-MS system at Adelaide Microscopy (The University of Adelaide, Adelaide, South Australia, Australia) following the standard protocols described in Gillanders (2002). Briefly, the samples were placed in the ablation chamber of the Agilent 8900 ICO-MS Triple Quad, after which the spots for ablation were selected. Two spots (30  $\mu$ m diameter) were selected per sample, one at the core of the otolith, and a second further towards the edge. Ablation was performed on all 297 samples for otolith edge analysis, but only 212 samples otolith core analysis. The decision to perform the ablation on a reduced number of samples for core analysis was made after considering our excessively large sample size, as well as both the additional time and financial cost required. The laser was fired at the selected spots, and <sup>7</sup>Li, <sup>23</sup>Na, <sup>24</sup>Mg, <sup>55</sup>Mn, <sup>65</sup>Cu, <sup>66</sup>Zn, <sup>88</sup>Sr, <sup>137</sup>Ba and <sup>208</sup>Pb isotopes from the ablated material were analysed. MACS (calcium carbonate material) and NIST 612 standards were used as internal reference standards for correction of variations of ablation yield and instrument drift respectively (Gillanders 2002). All sample concentrations for each element were found to be above the limit of detection. Mean estimates of precision (% CV, coefficient of variation)

based on our MACS standards were: 1.16% (Li), 0.35% (Na), 0.41% (Mg), 0.28% (Ca), 0.62% (Mn), 0.73% (Cu), 0.97% (Zn), 0.65% (Sr), 0.62% (In), 0.87% (Ba) and 0.31% (Pb). Mean percentage recovery based on the NIST 612 internal standard was 100% across all elements.

Data reduction was performed using the *lolite* package (Paton et al. 2011) for Igor Pro software environment. This was followed with estimation of element concentrations ratioed against Ca concentrations to obtain element: Ca ratios in µmol/mol. Canonical analysis of principal coordinates (CAP) was performed for a constrained ordination between locations to determine if there were any significant differences based on elemental signatures. Permutational multivariate analysis of variance (PERMANOVA) was used to further test the statistical effects of these differences. The leave-one-out cross validation (LOO) was performed to evaluate the performance of classification to each location (Wong 2015). All analyses were performed in PRIMER 7 and separately for otolith edge and core samples.

#### Acoustic telemetry

An acoustic array comprising of 37 listening stations was established across Corner Inlet and used in combination with an additional 20 offshore (i.e., outside Corner Inlet) receivers deployed in the region for a separate project, to track Rock Flathead movement patterns (Figure 2). The location of each station was strategically selected to maximise coverage across and around the inlet, ensuring all entrances were covered by a station to detect movement in and out of the inlet. Each station consisted of an acoustic receiver (Vemco VR2W-69 kHz or VR2Tx) attached to either a constructed mooring or existing navigational-aid markers by contracted commercial divers. This was done to maximise leverage of pre-existing infrastructure, with ease and efficiency of future servicing considered. Each receiver was first deployed between the 20<sup>th</sup> and 21<sup>st</sup> of November 2020 and serviced once between the 17<sup>th</sup> and 18<sup>th</sup> of October 2021. MNP\_02 (VR2W-106662) and MNP\_06 (VR2W-101759) were serviced on the 16<sup>th</sup> of January 2022 due to poor water visibility earlier which prevented the receivers from being located by the divers. The final round of receiver recovery occurred between the 10<sup>th</sup> and 11<sup>th</sup> of October 2022.

A total of 70 Rock Flatheads ranging between 27 and 57 cm in length, were surgically implanted with acoustic tags (Vemco V9-2x-BLU-1) across three tagging locations on 20 January 2021 (31 fish), 24 February 2021 (29 fish) and 15 June 2021 (10 fish). Each tag was 9mm in diameter, 27.5mm length and 4.5g weight in air (2.7g in water). Each fish was captured opportunistically by volunteer commercial fishers using either purse seine or gillnets. Upon capture, each fish was visually examined to ensure it was in an overall healthy condition to increase the likelihood of post-

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operational recovery. Selected fish were first transferred to a holding bin (plastic 80x40cm "nelly" bin with fresh seawater) before moving to a second anaesthetic bin dosed with AQUI-S anaesthetic (15-20mg/L). Once anaesthetised (i.e., upon loss of equilibrium), an acoustic tag was implanted internally following the methods described in Taylor et al. (2014). Briefly, a small incision was carefully made on the ventral cavity using a scalpel blade. Precaution was taken to ensure that no organs were punctured during this process. The acoustic tag was then inserted into the coelomic cavity, after which the incision was stitched up using either a one or two single suture knots, depending on the incision size. Following the surgery, each fish was placed in a recovery net hung alongside the stationary vessel. Upon recovery (i.e., when normal opercular activity was observed, generally within 5 minutes) the fish was released at the same location of capture.





Upon recovery of the receivers, both during the initial servicing and final retrieval, the raw data was downloaded using the Vemco User Environment (VUE) software (v. 2.8.1). The resultant raw detection and telemetry events files were stored in a local database and uploaded to the Australia Animal Acoustic Telemetry Database (https://animaltracking.aodn.org.au/). Data was explored using RStudio (v. 2022.12.0; R Core Team 2020) and the *remora* R-package (v 0.7.1) developed by the Integrated Marine Observing System (IMOS; Jaine et al. 2021).

### Results

#### **Population genomics**

A total of 125 individual Rock Flathead samples representing 7 sampling locations were successfully genotyped at 4,162 SNP loci, with 232 loci remaining for analysis following bioinformatic processing. The reduced number of loci is simply a function of low-density genome sequencing and species genome composition, although the number of loci retained is sufficient for analytical purposes of this study (Miller et al. 2026). Estimates of genetic diversity were moderate and relatively consistent across all sample locations, with allelic richness ranging from 1.30 to 1.41 and expected heterozygosity ranging from 0.18 to 0.23 (Table 2). A weak excess of heterozygotes was observed at 2 locations (CI and PPB), although across all locations inbreeding coefficients ( $F_{IS}$ ) did not differ significantly from zero indicating random mating (Table 2). A global estimate of population differentiation was found to be moderate-high and differed significantly from zero ( $F_{ST}$  = 0.09; 95% CI = 0.07 - 0.10) indicating limited gene flow and significant genetic structuring between sampling locations. Pairwise estimates of population differentiation indicate limited gene flow and significant genetic structuring between regions (Victoria, Tasmania, and Flinders Island), and between both Victorian sample locations (CI and PPB; Table 3). Conversely, a lack of significant genetic differentiation was observed for all pairwise estimates between Tasmanian sample locations, indicating a lack of genetic structure in this part of the sampling distribution.

**Table 2.** Statistics for Rock Flathead screened at seven sample locations with 232 SNP loci. Mean values over loci are presented for allelic richness (r), expected ( $H_E$ ) and observed ( $H_O$ ) heterozygosities, and inbreeding coefficients ( $F_{IS}$ ).

| Location | n  | r    | HE   | Hο   | <b>F</b> IS |
|----------|----|------|------|------|-------------|
| CI       | 33 | 1.41 | 0.23 | 0.26 | -0.07       |
| PPB      | 33 | 1.4  | 0.22 | 0.27 | -0.13       |
| FI       | 8  | 1.35 | 0.2  | 0.22 | -0.09       |
| М        | 5  | 1.3  | 0.18 | 0.17 | 0.01        |
| RC       | 9  | 1.33 | 0.19 | 0.18 | 0.03        |
| S        | 33 | 1.34 | 0.19 | 0.19 | 0.00        |
| W        | 4  | 1.32 | 0.19 | 0.2  | -0.06       |

|     | CI    | РРВ   | FI    | М     | RC    | S     | W |
|-----|-------|-------|-------|-------|-------|-------|---|
| CI  | *     |       |       |       |       |       |   |
| PPB | 0.032 | *     |       |       |       |       |   |
| FI  | 0.056 | 0.071 | *     |       |       |       |   |
| Μ   | 0.099 | 0.121 | 0.124 | *     |       |       |   |
| RC  | 0.108 | 0.123 | 0.118 | 0.000 | *     |       |   |
| S   | 0.099 | 0.112 | 0.105 | 0.010 | 0.012 | *     |   |
| W   | 0.064 | 0.096 | 0.083 | 0.004 | 0.011 | 0.009 | * |

**Table 3.** Pairwise estimates of genetic differentiation ( $F_{ST}$ ) among Rock Flathead sample locations. Values shown in bold are significant (P < 0.001) after 10,000 permutations and correction for multiple comparisons.

Site names and corresponding codes are as follows: CI (Corner Inlet), PPB (Port Phillip Bay),
 FI (Flinders Island), M (Montagu), RC (Rocky Cape), S (Stanley), W (Wynard)

AMOVA also showed significant differentiation between regions (Victoria, Tasmania, and Flinders Island), between populations within regions, as well as differences within populations. As expected, the majority of the genomic variation was explained by variation within populations (90.3%; P < 0.001), whereas variation between regions explained 7.9% (P < 0.001) of the variation, and populations within regions explained 1.8% (P < 0.001). The relationships between sample locations are best depicted by the density plot and two-dimensional biplot of SNP variation generated by DAPC and PCA, respectively (Figures 3 and 4). DAPC indicated two distinct population clusters, with Rock Flathead genotypes from Corner Inlet, Port Phillip Bay and Flinders Island assigned to one cluster and all Tasmanian genotypes assigned to the other (Figure 3). Further spatial resolution is provided in the PCA biplot with the two axes capturing almost 15% of the total variation (x-axis = 11.8%, y-axis = 3.0%; Figure 4). This plot indicates a clear separation of individuals from Tasmania, Victoria, and Flinders Island across the x-axis. The two Victorian sample locations (CI and PPB) are further separated along the y-axis, while all locations from Tasmania form a single admixed cluster. These patterns of variation among sample locations are consistent with the pairwise estimates of population genetic differentiation (F<sub>ST</sub>; Table 3). Finally, STRUCTURE analyses identified two distinctive population clusters (K = 2), separating Rock Flathead from Tasmania and mainland Australia, with Flinders Island appearing to be a mixed ancestral genotype (consistent with its geographical intermediate location; Figure 5). Analyses were repeated on mainland populations only, but unlike other analyses failed to detect genetic differentiation between Corner Inlet and Port Phillip Bay. This is consistent with STRUCTURE insensitivity in resolving finer-scale patterns of genetic structure which is reported widely in the literature.



**Discriminant function 1** 

**Figure 3.** Density plot of axis 1 eigenvalues based on discriminant analysis of principle components of 232 loci. Blue population cluster consists of Rock Flathead genotypes from Corner Inlet, Port Phillip Bay and Flinders Island, while the red population cluster consists of all Tasmanian Rock Flathead genotypes.



**Figure 4.** Two-dimensional scatter plot showing the relationships among Rock Flathead sample locations based on principle components analysis of 232 loci.



**Figure 5.** Bayesian STRUCTURE plot of the estimated membership coefficient (y-axis) for each individual in each of the two population clusters identified (indicated by the red and blue colourations). Each individual flathead is represented by a single vertical line broken into segments, where segments are proportional to the membership coefficient for each of the population clusters.

#### **Otolith microchemistry**

CAP results from otolith edge samples did not immediately reveal any obvious differences in chemical signals between sample regions (Figure 6). However, the PERMANOVA indicated significant differences between locations (*P* = 0.042; Table 4). The results from LOO indicated that 141 of 296 edge samples (47.64%) were correctly classified to their origins, with a mis-classification error of 52.36%. Overall, CI samples had the highest percentage agreement of sample allocation (64.84%; Table 5). In contrast, 36.21% of TAS samples were correctly allocated to their origin location, while both PPB and F showed low levels of origin allocation, 4.17% and 0% agreement, respectively (Table 3).



**Figure 6.** Canonical analysis of principal coordinates (CAP) ordination plot of Rock Flathead otolith edge samples based upon a Euclidean distance similarity matrix.

| Source   | df  | SS     | MS     | F      | P-value | Unique<br>perms |
|----------|-----|--------|--------|--------|---------|-----------------|
| Location | 3   | 12.702 | 4.234  | 2.0398 | 0.042   | 998             |
| Residual | 292 | 606.09 | 2.0757 |        |         |                 |

Table 4. PERMANOVA between locations for otolith edge samples

**Table 5.** Leave-one-out cross validation (LOO) allocation of observations to locations for otolith edge samples

| Original<br>group | TAS | CI  | РРВ | FI | Total n | % correct |
|-------------------|-----|-----|-----|----|---------|-----------|
| CI                | 55  | 118 | 4   | 5  | 182     | 64.84     |
| РРВ               | 21  | 20  | 2   | 5  | 48      | 4.17      |
| FI                | 5   | 3   | 0   | 0  | 8       | 0.00      |
| TAS               | 21  | 23  | 4   | 10 | 58      | 36.21     |

• Site names and corresponding codes are as follows: CI (Corner Inlet), PPB (Port Phillip Bay), FI (Flinders Island), TAS (Montagu, Rocky Cape, Stanley, Wynard)

CAP results from otolith core samples once again did not immediately reveal any obvious differences in chemical signals between locations (Figure 7), but with PERMANOVA indicating significant differences in otolith microchemistry profiles among sample locations (*P* = 0.004; Table 6). The results from LOO indicated that 102 of 207 core samples (49.28%) were correctly classified to their origins, with an overall mis-classification error of 50.72%. Again, CI samples had the highest percentage agreement of sample allocation (58.95%; Table 7) followed by TAS (57.90%; Table 7), with PPB and F performed having comparatively lower allocation percentages (23.40% and 25.00% agreement, respectively, Table 7).



**Figure 7.** Canonical analysis of principal coordinates ordination (CAP) plot of otolith core samples based upon a Euclidean distance similarity matrix.

| Table 6. PERMANOVA | between locati | ions for otolith | core samples |
|--------------------|----------------|------------------|--------------|
|--------------------|----------------|------------------|--------------|

| Source   | d <i>f</i> | SS     | MS     | F      | P-value | Unique<br>perms |
|----------|------------|--------|--------|--------|---------|-----------------|
| Location | 3          | 12.544 | 4.1812 | 3.1714 | 0.004   | 999             |
| Residual | 203        | 267.64 | 1.3184 |        |         |                 |

| Original<br>group | TAS | CI | РРВ | FI | Total | % correct |
|-------------------|-----|----|-----|----|-------|-----------|
| CI                | 16  | 56 | 11  | 12 | 95    | 58.95     |
| РРВ               | 19  | 11 | 11  | 6  | 47    | 23.40     |
| FI                | 1   | 3  | 2   | 2  | 8     | 25.00     |
| TAS               | 33  | 7  | 13  | 4  | 57    | 57.90     |

**Table 7.** Leave-one-out cross validation (LOO) allocation of observations to locations for otolith core samples

• Site names and corresponding codes are as follows: CI (Corner Inlet), PPB (Port Phillip Bay), FI (Flinders Island), TAS (Montagu, Rocky Cape, Stanley, Wynard)

#### Acoustic telemetry

Nine out of 70 tagged Rock Flathead were detected across 10 different acoustic stations (Figure 8) between 19 March 2021 and 26 September 2022, resulting in a total of 1,912 acoustic detections across all receivers. Four of the nine fish (A69-1602-41367, 41378, 41402 and 41405) were detected across multiple receivers, while the remaining five fish were detected at single stations only. Of the nine detected fish, three were tagged at tagging location 1 (A69-1602-41363, 41367 and 41378), four were tagged at tagging location 2 (A69-1602-41402, 41404, 41405 and 41412), and two were tagged at tagging location 3 (A69-1602-60002 and 60003). Refer to Figure 9 for detail on the tagging locations and locations of the acoustic stations within Corner Inlet.



**Figure 8.** Detections of individual Rock Flathead between the 19<sup>th</sup> of March 2021 and 26<sup>th</sup> of September 2022. Coloured spots correspond to the different acoustics stations at which fish was detected. Station name codes are as follows: CI (Corner Inlet, outside Marine National Park), MNP (Corner Inlet, inside Marine National Park).



Figure 9. Map of fish tagging locations and acoustic stations where Rock Flathead was detected on the receiver

We failed to recover nine receivers across this study. CI\_08, CI\_10, CI\_11 and MNP\_02 were lost during the servicing round. The most probable explanation for this would be due to the extreme weather activity experienced in the area. Strong winds, strong underwater currents, and poor visibility at the time of diving were all contributing factors to the failure to retrieve these receivers. During the dive, there were multiple instances where the diver was able to locate the mooring and/or attachment underwater, but the receiver appeared to have snapped off (indications such as broken cable ties was observed) and was no longer present. For the same reason, receivers were retrieved but not re-deployed at a further eight stations (CI\_07, CI\_17, CI\_18, CI\_21, CI\_22, CI\_23, PA\_04 and PA\_05) within the Corner Inlet array. This decision was made to minimise the loss of equipment at locations where we did not feel confident of retrieving them again. (CI\_19), (CI\_24), (MB\_01), (MB\_02) and (RB\_02) were lost during the final retrieval. We were advised by Port management that the navigational aids we attached the receivers to were repositioned by towing to their new sites. It was likely that the receivers were lost during this process.

### Discussion

In this study we used a combination of population genomics, otolith microchemistry and acoustic telemetry to characterise the biological connections among Rock Flathead populations spanning south-eastern Australia. The study focused specifically on the Corner Inlet Rock Flathead fishery, the region's only commercial fishery which has suffered notable declines, and where information on stock structure is needed to inform the sustainable management. Evidence from all three methods point to limited biological connections among Rock Flathead populations from south-eastern Australia, with the Corner Inlet Fishery appearing to be an isolated, self-recruiting stock. These findings challenge current assumptions of stock admixture based on contrasting findings from related species (i.e., Dusky Flathead; Taylor et al. 2020) and anecdotal reports of Rock Flathead dispersal patterns. Below we discuss the findings from this study in detail, including implications for future management of Rock Flathead fishing stocks at Corner Inlet and south-eastern Australia generally.

#### Evidence of limited stock connectivity

We provide multiple lines of evidence pointing to weak biological connections between most stocks across the study area. Findings from our population genomic analyses indicate Rock Flathead stocks from Corner Inlet and Port Phillip Bay to be genetically distinct entities, suggesting that gene

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flow/interbreeding between these embayment/inlet populations and those from open coastal habitats is likely to be limited. Furthermore, we also found Rock Flathead from Flinders Island to be genetically distinct and isolated from stocks from the Australian mainland and Tasmanian Rock Flathead populations. While findings should be interpreted with some degree of caution due to the comparatively small sample numbers, it is plausible that the deeper waters around Flinders Island act as barrier to dispersal and gene flow given the species' preference for shallow inshore waters (CSIRO Marine & Atmospheric Research & Bray, 2020). In contrast, we detected a lack of significant genetic differentiation between Rock Flathead from four different locations from northern Tasmania. However, this is also a plausible finding given the short distances between sample locations (range 40 – 100 kms) and the connected nature of these open coastal habitats.

Results from the otolith microchemistry analyses support the population genetic data by revealing a distinctive microchemistry profile for the Corner Inlet fishing stock, again pointing to stock isolation. Importantly, these patterns are consistent across both edge and core otolith samples, which are presentative of the fish's early life stage and moment of capture respectively (de Almeida et al. 2024). This provides evidence for limited dispersal and habitat usage beyond Corner Inlet for both juvenile life and adult Rock Flathead. In contrast, we observed little variation in otolith microchemistry profiles among fish from all remaining sample locations (Port Phillip Bay, Flinders Island and Tasmania). Given the results from the genetic analyses (described above) and the acoustic telemetry (described below) it is unlikely that this homogeneous pattern reflects stock admixture. Instead, this may point to limitations in detecting significant differences among marine habitats on these spatial scales where chemistry gradients are likely to be less pronounced. In comparison, Corner Inlet is an estuarine environment, where the chemistry of the local environment is expected to differ from marine environments, hence our ability to detect differences in otolith microchemistry profiles in this instance.

Finally, acoustic telemetry showed no indications that tagged individuals were leaving Corner Inlet throughout the entire study period. Detections from nine out of 70 tagged individuals (12.86%) were limited to receivers within the inlet, with majority of detections occurring within the Marine National Park (MNP). Furthermore, there were no Rock Flathead detections at any of the 20 offshore receivers deployed outside the inlet for a separate project (Figure 2), which provides further evidence that movement out of the inlet is unlikely. Though we only received detections from a small number of tagged fish, this provides further evidence that Rock Flathead have small home ranges. In order to leave the inlet, tagged fish would have to pass multiple receivers. Therefore, it is most likely that the lack of detections reflects limited fish movement. This is further

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supported by the movement of fish detected on multiple receivers, which were typically limited to adjacent receivers separated by short distances.

Overall, our telemetry results further support our population genomics and otolith microchemistry findings, suggesting that dispersal appears to be limited for this species. However, the loss of receivers at Cl\_08, Cl\_10 and Cl\_11, which were positioned along the main opening to the inlet, was not ideal and must be acknowledged as a limitation. The total loss and early retrieval of receivers may also be a contributing factor to the limited number of detections. It is important to also consider other limitations of acoustic telemetry studies. Post-surgery survival of tagged individuals cannot be monitored in the wild, and we are also uncertain if the presence of an internal tag may affect natural behaviour in any way (Crossin et al. 2017). Detection range of receivers is also highly dependent on environmental influences and in regions that experience extreme weather variability, such as Corner Inlet, receivers tend to have reduced detection ranges (Crossin et al. 2017). This may result in lower number of detections even if a tagged individual is present within the area. False-positive detections, known as collisions, may also occur if a frequency produced by ambient noise matches the same code of a tagged fish (Crossin et al. 2017). This is particularly a concern for the six individuals that have very limited detections (i.e., single detections) if such movement does not match the general behaviour of the species. In the case of the Rock Flathead, being an ambush predator means it is likely to spend most of its time at the same location (Coulson et al. 2015), which makes single detections unlikely.

Interestingly, our findings are inconsistent with those from previous studies from a sister species with an overlapping distribution, Dusky Flathead (*P. fuscus*). A previous genetic study on Dusky Flathead indicated little genetic structure and admixture across the fishery spanning multiple estuaries and more than 500 kms of the New South Wales Coastline (Taylor et al. 2020). However, a tag and recapture study showed that movement of adult Dusky Flathead among estuaries was limited, with over 90% of tagged fish being recaptured within the same estuary (Gray and Barnes 2015). Consequently, Taylor et al. (2020) suggested that population admixture in Dusky Flathead is most likely to be driven by the dispersal of eggs and larvae over large spatial large scales. Despite the limitations of our acoustic telemetry study, our finding also pointed to limited adult movement beyond Corner Inlet. However, evidence of significant genetic structuring in Rock Flathead suggests that egg and larval dispersal is also likely to be limited to local spatial scales. The reproductive biology and early life-history of Rock Flathead are presently not well understood. Sand Flathead, a closely related species to the Rock Flathead, was found to have pelagic larval stages, with dispersal mostly limited to inshore areas within proximity of known spawning sites in Tasmania (Jordan 2001).

If Rock Flathead exhibits similar patterns of larval dispersal, this will further support our inference that the Corner Inlet population is isolated and may even suggest that spawning occurs locally within the inlet. Future studies focused on gaining a better understanding of reproduction and early lifehistory, coupled with larval dispersal models may be helpful to prove this hypothesis.

#### Implications for management

Our findings have implications for several areas of Rock Flathead management at Corner Inlet. Importantly, our data sources point to the Corner Inlet Rock Flathead being an isolated, selfrecruiting stock, which is an important consideration when gauging the vulnerability of the fishery to risks of overexploitation, as well environmental disturbances, and the potential for natural recovery following depletion events. Recruitment dynamics within the fishery are likely to be heavily dependent on the local adult population, with recruitment from non-local sources being limited. Consequently, total allowable catch (TAC) and size limits should be set appropriately to avoids risk of depleting the local reproductive adult population and to ensure ongoing recruitment and fishery sustainability.

Managers should also pay careful attention to the condition of local habitats that are expected to be key for sustaining both adult and juvenile fish populations within the inlet. For example, seagrass habitats are recognised as important nursery grounds for juvenile Rock Flathead but have suffered significant declines in Corner Inlet in recent decades, largely due to catchment processes (Ford, Barclay & Day 2016). The integrity of local nursery grounds is likely to be key to the long-term viability of the fishery, with declines of seagrass meadows being a potential contributing factor to historical declines in the fishery (Ford, Barclay & Day 2016). Consequently, local seagrass restoration initiatives are likely to benefit the fishery.

The isolated nature of the Corner Inlet Rock Flathead stock also means it has limited potential for natural recovery following major depletion events, compared to large, admixed fisheries where recruitment from non-local sources can contribute to fishery recovery. While we have seen that the local Rock Flathead stock has recovered following the declines of the mid 90s, translocations may be needed to assist the recovery of the fishery if future depletion events occur, especially in more severe circumstances. In recent years there have been several investigations into the feasibility of flathead restocking programs in Victoria, including at Corner Inlet (Ingram, 2019). However, given the isolation of the Corner Inlet stock, careful consideration should also be paid to the genetic composition of animals used for restocking activities, to preserve the genetic integrity and health of the local fishery. Strategic brood stock selection could also play a key role in enhancing the resilience of the fishery to rapid environmental changes (Hoffmann, Miller & Weeks 2021).

Finally, the findings from the current study have implications for fisheries biosecurity. Many fisheries around Australia and overseas are impacted by disease, including pathogens that are both horizontally and vertically transmitted (Arulmoorthy et ak. 2020; Zainathan & Knowles 2022). While knowledge of existing diseases in flathead species is limited (with the exception for bacterial kidney disease; Traxler and Bell, 1988), the isolation of the Corner Inlet stock suggests there is likely to be a low risk of the spread of novel diseases to or from the fishery in the absence of human vectors. However, restricted movement also means that spread of disease resistance alleles is also likely to be spatially limited, but the evolution of resistance within Corner Inlet Fishery may occur relatively quickly given the small, isolated nature of the fishery.

# Conclusion

The primary objective of this study was to determine the population structure of Rock Flathead in south-eastern Australia, with emphasis on understanding how the Corner Inlet population relates to other populations. Secondly, we aimed to quantify the movement and residency patterns of Rock Flathead in Corner Inlet to help inform future stock assessment and sustainable fisheries management. This study has used a suite of complementary analytical tools, which all point to the Corner Inlet Rock Flathead being a genetically distinct and biologically isolated fishing stock. This suggests the fishery is a self-recruiting entity and that any recruitment from non-local sources is expected to be minimal. Although our telemetry study was compromised by some issues, fish tracks indicate highly localised movements and high degrees of fish residency within Corner Inlet. These findings are consistent with the population genomic work which indicated a signal of genetic uniqueness, and otolith microchemistry profiles which indicate restricted movement and local habitat use within the Inlet across both juvenile and adult life stages. In conclusion, we advise that future management of this fishery considers the isolated nature of the fishery when conducting stock assessments, setting catch limits and gauging risks for environmental disturbance. Detailed discussion of the implications of the findings of this study in the context of future fisheries management is provided in the discussion section of the report. This new information will provide a much-needed resource to assist managers in future stock assessments and sustainable management of the Corner Inlet Rock Flathead fishery.

### Recommendations

The future management of this fishery should consider the isolated nature of the Corner Inlet Rock Flathead fishery when conducting stock assessments, setting catch limits and gauging risks for environmental disturbance, specifically:

1. Total allowable catch (TAC) and size limits should be set appropriately to avoids risk of depleting the local reproductive adult population and to ensure ongoing recruitment and fishery sustainability.

2. Managers should also pay careful attention to the condition of local habitats that are expected to be key for sustaining both adult and juvenile fish populations within the inlet. For instance, conservation and restoration of seagrass meadows habitats (important nursery habitats) is likely to benefit the fishery.

3. Translocations may be needed to assist the recovery of the fishery if future depletion events occur, especially in more severe circumstances. In such cases, careful consideration should be paid to the genetic composition of animals used for restocking activities, to preserve the genetic integrity and health of the local fishery.

4. The isolation of the Corner Inlet stock suggests there is likely to be a low risk of the spread of novel diseases to or from the fishery in the absence of human vectors.

#### **Further development**

Reflection on the findings and limitations of this study highlight the value of additional research investments. In particular, high levels of genetic structuring across the species range, and localised movement patterns / site fidelity from acoustic telemetry, suggests the possibility of structuring within Corner Inlet itself. Consequently, replicated spatial sampling within Corner Inlet would help to rule out the possibility of further population subdivision. Overall, the genomic and otolith microchemistry components were sound and provided clear outputs that point to the isolation of the Corner Inlet Fishery. Unfortunately, there were some issues with the acoustic telemetry work, and repeat studies would help to gain a better picture of the extent of fish movement within Corner Inlet and patterns of residency / habitat use. Reflecting upon our experience, the mooring design was not well suited for local environmental conditions, which led to unfortunate loss of receivers. Also, given we were unable to determine post-surgery survival we cannot confidently determine if the failure to detect some tagged animals is due to highly localised movement patterns or due to post-surgery mortality, therefore understanding the risk of this surgery to fish health and behaviour would assist our data interpretation. Future research might benefit from increasing array coverage around the inlet, and potentially using "wandering" receivers to determine shorter-term movement patterns.

## **Extension and Adoption**

This project was undertaken in direct partnership with the end-user, the industry representatives (stakeholders and fishers) of Corner Inlet Rock Flathead fishery. Industry representatives played a key role on the collection of biological samples, deployment of acoustic listening stations and catching and tagging local Rock Flathead with acoustics tags. A workshop with the industry partners will be held to communicate all results and recommendations for future management. The final FRDC report will be shared with all Rock Flathead fishery stakeholders, and the results will be disseminated to the wider scientific community through a student thesis and three peer-reviewed publications which we intend to submit for review in 2025.

#### **Project coverage**

This report represents the only written description of the project findings. This project, funded by the FRDC, supports a Higher Degrees PhD student at Deakin University, and all research content is expected to be presented in the form of a thesis and peer-reviewed publications.

An article was published in FRDC Fish News, "<u>Research into the dynamics of Rock Flathead</u> (*Platycephalus laevigatus*) in Victoria's Corner Inlet fishery has provided surprising new insights into the species which will better inform future fisheries management", December 2024.

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