

# Natal origin of Golden Perch and Murray Cod across northern Victorian rivers

Z. Tonkin, A. Harris, B. Fanson, C. Bice, D. Dawson, G. Hackett, W. Koster, K. Krusic-Golub, J. Lieschke, A. Sadekov, A. Strawbridge, A. Wainwright and B. Zampatti

March 2026

Arthur Rylah Institute for Environmental Research Published Report



Arthur Rylah Institute for Environmental Research  
Department of Energy, Environment and Climate Action  
PO Box 137, Heidelberg, Victoria 3084  
Phone (03) 9450 8600  
Website: [www.ari.vic.gov.au](http://www.ari.vic.gov.au)

**Citation:** Tonkin, Z., Harris, A., Fanson, B., Bice, C., Dawson, D., Hackett, G., Koster, W., Krusic-Golub, K., Lieschke, J., Sadekov, A., Strawbridge, A., Wainwright, A. and Zampatti, B. (2026). *Natal origin of Golden Perch and Murray Cod across northern Victorian rivers*. Published Report for the Water and Catchments Group, Department of Energy, Environment, and Climate Action. Arthur Rylah Institute for Environmental Research, Department of Energy, Environment and Climate Action, Heidelberg, Victoria.

**Front cover photo:** Adult Golden Perch (top) and juvenile Murray Cod (bottom; images ARI).

ISBN 978-1-76176-790-6 (pdf/online/MS word)

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We honour Elders past and present whose knowledge and wisdom has ensured the continuation of culture and traditional practices.

DEECA is committed to genuinely partnering with Victorian Traditional Owners and Victoria's Aboriginal community to progress their aspirations.



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# **Natal origin of Murray Cod and Golden Perch recruits across northern Victorian rivers**

**Zeb Tonkin<sup>1</sup>, Annique Harris<sup>1</sup>, Ben Fanson<sup>1</sup>, Chris Bice<sup>2</sup>, David Dawson<sup>1</sup>, Graeme Hackett<sup>1</sup>, Wayne Koster<sup>1</sup>, Kyne Krusic-Golub<sup>3</sup>, Jason Lieschke<sup>1</sup>, Aleksy Sadekov<sup>4</sup>, Aaron Strawbridge<sup>2</sup>, Ashlea Wainwright<sup>5</sup> and Brenton Zampatti<sup>6</sup>**

<sup>1</sup>Arthur Rylah Institute for Environmental Research Department of Energy, Environment and Climate Action  
123 Brown Street, Heidelberg, Victoria 3084

<sup>2</sup>Inland Waters and Catchment Ecology Program, South Australian Research and Development Institute (SARDI) – Aquatic Sciences, PO Box 120, Henley Beach, SA 5022, Australia

<sup>3</sup>Fish Aging Services Pty Ltd., Queenscliff, Victoria, 3223, Australia

<sup>4</sup>Ocean Graduate School, The University of Western Australia, Perth, WA 6009, Australia.

<sup>5</sup>School of Geography, Earth & Atmospheric Sciences, The University of Melbourne, Victoria 3010 Australia

<sup>6</sup>Environment Research Unit, Commonwealth Scientific and Industrial Research Organisation (CSIRO), Waite Campus, Glen Osmond, SA 5064, Australia

Arthur Rylah Institute for Environmental Research  
**Published Report for Water and Catchments, DEECA**  
**Department of Energy, Environment and Climate Action**

# Acknowledgements

We thank the Water and Catchments group of the Department of Environment, Energy and Climate Action (DEECA) for funding this work. Data for the Murray River and some samples from the Goulburn River was provided through the Commonwealth FLOW MER program (Commonwealth Environmental Water Holder) and River Murray Monitoring Program (funded by the Commonwealth Environmental Water Holder and The Living Murray Joint Venture). Thanks to Scott Raymond, Adrian Kitchingman, Andrew Pickworth and Ben Iscaro (Arthur Rylah Institute, ARI) for collection of fish data Frank Amtstaetter and Pam Clunie from the Arthur Rylah Institute provided useful comments on the draft version of this document. We also thank the Victorian Fisheries Authority, particularly Rhiannon Atkinson and Mark Adams, for provision of detailed stocking information. Sampling in Victoria was completed under Victorian Flora and Fauna Guarantee Permit 10010854, Fisheries Victoria Research Permit RP-827. Sampling in NSW waters was conducted under Industry and Investment NSW Fish Collection Permit FP24/37 and FP23/42. Animal Care and Ethics Approval was granted under the Victorian DEECA ethics permit AEC 24-003.

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# Summary

## Context and aim

Riverine fish populations in the Murray–Darling Basin (MDB) have experienced significant declines, largely due to overfishing, altered flow regimes, habitat degradation, and barriers to movement. Failure of natural recruitment is a key driver of these declines, prompting interventions such as environmental water delivery, habitat restoration, and stocking of hatchery reared fish. Indeed, major intervention efforts, including Commonwealth and State environmental water delivery programs, specifically target fish recruitment as an objective (e.g. fish recruitment is a specific target embedded within the Murray–Darling Basin Plan). Therefore, monitoring and evaluation programs traditionally focus on abundance and size structure of fish populations to report against such objectives. Information on natal origin—whether fish are wild recruits or stocked—however remains limited. This study addresses that gap using otolith microchemistry to determine the natal origin of Murray Cod *Maccullochella peelii* and Golden Perch *Macquaria ambigua*, two species of conservation, cultural and recreational value, across key waterways of northern Victorian.

## Methods

Natal origin of Murray Cod and Golden Perch was determined using otolith microchemistry ( $^{87}\text{Sr}/^{86}\text{Sr}$  ratios) across major northern Victorian waterways subject to stocking or environmental water delivery. Otoliths were collected from 374 juvenile Murray Cod (<180 mm total length; primarily age 0+ and 1+) sampled during routine monitoring between 2017 and 2025 from 12 northern Victorian rivers and creeks, with additional otoliths obtained from adult Murray Cod salvaged during a 2022 fish death event in Broken Creek. To characterise hatchery signatures, otoliths were analysed from 148 Murray Cod fingerlings sourced from hatcheries used to stock Victorian waterways over the study period, with hatchery water samples analysed where fingerling otoliths were unavailable. For Golden Perch, otoliths were collected from 341 individuals (juveniles and adults) sampled during routine monitoring in 2024 and 2025 from Victorian tributaries and multiple reaches of the Murray River. These were compared with previously published otolith data collected in 2018 due to major floods, environmental water delivery and an increase in the number of stocked fish released. Natal origin of Murray Cod was inferred by comparing otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$  values with cohort-specific waterway and hatchery signatures using simulation-based best-match approaches. Golden Perch natal origin was assessed using a Bayesian assignment model that compared otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  profiles with an established MDB isoscape to classify individuals as locally recruited, migrant or hatchery-origin. Results were summarised across waterways and cohorts to assess spatial and temporal variability in recruitment sources.

## Results

### Murray Cod

There was substantial spatial variation in recruitment sources across northern Victorian waterways. The Goulburn, Broken and Murrumbidgee systems consistently showed a high contribution of wild-origin Murray Cod, with individual cohorts typically dominated by naturally spawned fish (~75–100% wild-origin), despite ongoing stocking. In contrast, systems such as Gunbower Creek and the Campaspe River exhibited mixed recruitment, with cohorts comprising varying contributions of wild-origin individuals through time. Conversely, the highly modified and cold-water impacted Mitta Mitta River supported populations largely dominated by fish of hatchery origin, with wild-origin fish detected in only a limited number of cohorts. In several smaller or more heavily stocked waterways, interpretation was constrained by limited sample sizes and overlap between hatchery and waterway  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures, resulting in a higher proportion of fish assigned as unknown origin.

### Golden Perch

Golden Perch populations showed mixed natal origin through both space and time, reflecting the combined influence of large-scale recruitment events, longitudinal connectivity and stocking. Compared with the 2018 assessment, the 2024–25 sample showed a slightly higher overall contribution of wild-origin and migrant fish, and a corresponding reduction in the proportion of stocked individuals. Across all systems sampled in 2024–25, naturally recruited fish comprised approximately ~62% of individuals, with the remaining ~38% of hatchery origin. Of the naturally recruited component, most fish originated from the Murray River (~31% combined lower, mid and upper Murray), followed by the lower Darling (~15%) and the Goulburn–Broken system (~7%). The mid and lower reaches of the Murray River were dominated by wild-origin and migrant fish, with stocked individuals typically contributing <10% of sampled fish. In contrast, Victorian tributaries

showed a much greater contribution of fish of stocked and migrated origin. The Goulburn River contributed a disproportionately large number of recruits to adjacent tributaries and upper Murray River reaches, despite limited previous evidence of its regional importance as a recruitment source.

#### *Limitations*

Interpretation of natal origin was constrained in some systems by overlap between hatchery and waterway  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures, incomplete hatchery reference data and low sample sizes in certain waterways or cohorts. These factors limited the ability to confidently assign natal origin for all individuals, particularly in recently stocked systems supplied by multiple hatcheries.

#### **Conclusions**

Overall, these results demonstrate that natural recruitment remains an important, though variable, contributor to Murray Cod and Golden Perch populations across northern Victorian waterways, thereby filling an important knowledge gap. Systems exhibiting mixed recruitment highlight the capacity for natural spawning to occur intermittently when flow, connectivity and habitat conditions are favourable, even in regulated environments. These findings can also inform the development or refinement of realistic flow-management objectives. For Golden Perch in Victorian tributaries outside the Goulburn River, recruitment to the broader metapopulation is limited, meaning management should prioritise connectivity, immigration cues from the Murray River, and maintenance of suitable baseflows to support habitat for these and stocked fish. Further interpretation of these results with other monitoring sources in relation to environmental drivers—particularly hydrological conditions—is required to better inform environmental flow planning, evaluation and adaptive management in northern Victorian waterways. Incorporating natal origin information alongside routine demographic monitoring and population genetics will strengthen interpretation of recruitment and population dynamics, thereby supplementing reporting against environmental water targets (including Basin Plan objectives). Nevertheless, differentiating wild from hatchery-origin fish is becoming increasingly difficult due to growing stocking complexity, meaning clearer hatchery sourcing and coordination and/or complementary data (e.g., genetics, demographics, spatial patterns) are needed to reliably interpret recruitment dynamics.

# Introduction

Riverine fish populations have declined globally, due to factors including overfishing, degraded habitat, barriers to fish passage and pollution (Cadwallader 1978; Cadwallader 1986; Koehn et al. 2014). Failure of natural recruitment is a key cause of population decline in riverine fishes globally and in (Birnie-Gauvin et al. 2017) and in Australia (King et al. 2009; Koehn et al. 2014; PIRSA 2015). A number of recovery interventions, including environmental water delivery, habitat restoration, protection of spawning stocks and the release of hatchery-reared fish (stocking) are often used to address poor recruitment and declining fish populations. Monitoring and evaluation are also important components of such recovery programs, both to assess outcomes and inform adaptive management of future interventions.

DEECA's water programs require information on fish populations to inform a range of management and policy needs. This includes evaluation of responses to management actions (especially environmental water), to meet Victoria's reporting obligations under the Murray-Darling Basin Plan (MDBP). Much of the information about fish populations required to meet DEECA's water programs needs comes from conventional population census surveys which record demographic measures such as catch and size structure, thereby facilitating estimates of distribution, relative abundance and recruitment. Such measures form part of foundational reporting requirements directly related to MDBP objectives, centred around increasing population size and improving structure (via successful recruitment) of priority native fish populations (e.g. Tonkin et al. 2024). The monitoring programs provide essential information on abundance and population structure, but recruitment data—which are also required—is often limited. This is particularly the case for priority species that are stocked or migrate between reaches to influence population dynamics, whereby without a clear understanding of whether fish are stocked or have moved into a particular area, it's challenging to interpret specific responses to management interventions. Nevertheless, complementary data collected as part of these monitoring programs can, and have previously been used to, fill such knowledge gaps. For example, genetic or otolith samples collected from fish as part of the Victorian Environmental Flow Monitoring and Assessment Program (VEFMAP) and Native Fish Report Card Program were used prior to 2020 to assess age structure, identify natal origin (stocked vs naturally recruited individuals) and assess movement of two native species, Murray Cod *Maccullochella peelii* and Golden Perch *Macquaria ambigua*, across the southern MDB (e.g. Harris et al. 2020; Zampatti et al. 2019; Harrison et al. 2025). A previous assessment of Murray Cod natal origin within five waterways subject to stocking showed the contribution of in-situ recruitment versus stocked individuals varied within and among waterways. This ranged from Murray Cod in the regulated and cold-water impacted Mitta Mitta River being largely supported by stocking (see Tonkin et al. 2023) to in-situ recruits dominating the population structure in the lower Goulburn River, despite significant stocking efforts (Tonkin et al. 2019; Harris et al. 2020). Previous assessments of Golden Perch indicate Victorian tributaries and the upper Murray reaches are largely dominated by fish of stocked origin or that have migrated from natal sources in the mid – lower Murray and Darling rivers, with some in-situ recruitment occurring in the Goulburn River (Zampatti et al. 2019).

This project repeats the otolith component of previous stages of the VEFMAP and Commonwealth Environmental Water Holder's Monitoring, Evaluation and Research Program (Flow-MER) to generate contemporary information on Murray Cod natal origin and Golden Perch age structure and natal origin in priority northern Victorian rivers (and reaches of the Murray River). Specifically, the delineation in dissolved strontium isotope ratios ( $^{87}\text{Sr}/^{86}\text{Sr}$ ) in water samples from across rivers or fish hatcheries of the Murray-Darling Basin (MDB) was related to otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  to reconstruct natal origin (e.g. Zampatti et al. 2019; Harris et al. 2020; Thiem et al. 2022; Zampatti et al. 2022). Since 2018 (period since previous otolith collections and assessments for Murray Cod and Golden Perch), northern Victorian waterways and the Murray River have been subject to considerable variation in river flows (including the largest flood event in more than 50 years), environmental water delivery and stocking efforts. For Golden Perch there was also a significant recruitment event in the lower Murray and Darling rivers, particularly the 2021/22 cohort, with the contribution of this cohort to upstream Victorian waterways unclear. Thus, data to indicate natal source and origin of fish provides considerable value to the annual demographic monitoring (distribution, abundance and size structure) by providing insights into the population structure and dynamics and how to best target management actions to maintain these populations. In addition, this information also contributes to broader collaborative projects that report on natural recruitment, natal origin and age structure, specifically the Whole of Murray River Assessment project and Area Scale MER for the Goulburn River and northern Victorian tributaries (Commonwealth Environmental Water Holder and CSIRO).

# 1 Methods

## 1.1 Study species and otolith collection and preparation

This study assessed natal origin of two high priority native fish species, Murray Cod and Golden Perch across priority northern Victorian waterways and for Golden Perch in the Murray River. Both species are endemic to the MDB, and have immense cultural, conservation and recreational value (Lintermans 2023). They are long-lived (>20 years) and remain widely distributed throughout most of their natural range but have experienced considerable declines in abundance (Lintermans 2023). To recover populations, both species are targeted with a range of interventions, particularly habitat restoration and flow management (including environmental water delivery) aimed at enhancing key life history processes such as movement and recruitment. Indeed, in-situ recruitment - particularly for Murray Cod, and movement - particularly for Golden Perch, are two key drivers of population dynamics of both species in northern Victorian waterways and the connecting Murray River (see Zampatti et al. 2019; Lyon et al. 2021). Enhancing both processes, to ultimately maintain or improve populations, is a key objective of the Basin Plan.

Stocking of hatchery produced fingerlings has also increased dramatically in recent years, with more than 2.9 million Murray Cod and 3 million Golden Perch released across Victoria in 2025 alone (<https://vfa.vic.gov.au/recreational-fishing/fish-stocking/db/search-species>). While the majority of these releases occurred in lakes and reservoirs for recreational fishing purposes, many liberations occurred in creek and riverine habitats subject to other interventions such as flow management. Assessing in-situ recruitment using conventional population surveys relies largely on detecting early life stages, particularly young-of-the-year (YoY). Considerable uncertainty arises however in those reaches that are stocked with captive bred fingerlings or are connected to adjoining reaches.

Otolith samples of both species were sourced from fish collected during routine monitoring (e.g. Tonkin et al., 2024) and, in the case of Murray Cod, in the Broken Creek, from 44 fish retrieved from a hypoxia induced fish kill event. For Murray Cod (with the exception of the aforementioned Broken Creek sample), samples were obtained from 371 juvenile fish 40–180 mm total length, and either 0+ (YoY) or 1+ years of age, with collections made annually (if present) from 2017–2025 (Table 1). Additional otoliths were obtained from fingerlings (n=148) sourced from all of the key hatcheries used to stock Murray Cod across Victoria (see analysis below). For Golden Perch, otoliths were collected from 341 individuals (juveniles and adults) sampled during routine monitoring in 2024 and 2025 from Victorian tributaries and multiple reaches of the Murray River (n=341; see Table 2

) and compared with data derived from samples collected from the same reaches in 2018 (n=240; see Zampatti et al. 2019). Comparisons were made with the 2018 sample due to major floods, environmental water delivery and an increase in the number of stocked fish released since this sample period.

Sagittal otoliths from all fish were removed and prepared for ageing and LA-ICPMS using transverse sections, incorporating the otolith core. Individual transverse sections were mounted collectively on 'master' slides using a thin layer of casting resin, and each section was polished using wetted lapping film (9 mm; see Zampatti et al. 2022).

## 1.2 Study reaches

This study used fish sampled from 13 waterways: the Broken, Campaspe, Goulburn, Loddon, Lindsay, Kiewa, Murray and Mitta Mitta rivers as well as the Pyramid, Broken, Mullaroo, Cudgewa and Gunbower creeks (Figure 1). Each of the Victorian waterways have been stocked by the Victorian Fisheries Authority (VFA) over the past years (<https://vfa.vic.gov.au/recreational-fishing/fish-stocking/db/search-species>), and the Murray River (sampled from Yarrawonga to the South Australian lower Murray) is an important source of migrant Golden Perch for the other rivers (Zampatti et al. 2019). We note, reaches of the Ovens, King and Murray rivers were not included for the Murray Cod assessment given they are not stocked, with juvenile fish captured highly likely to be in-situ recruits.

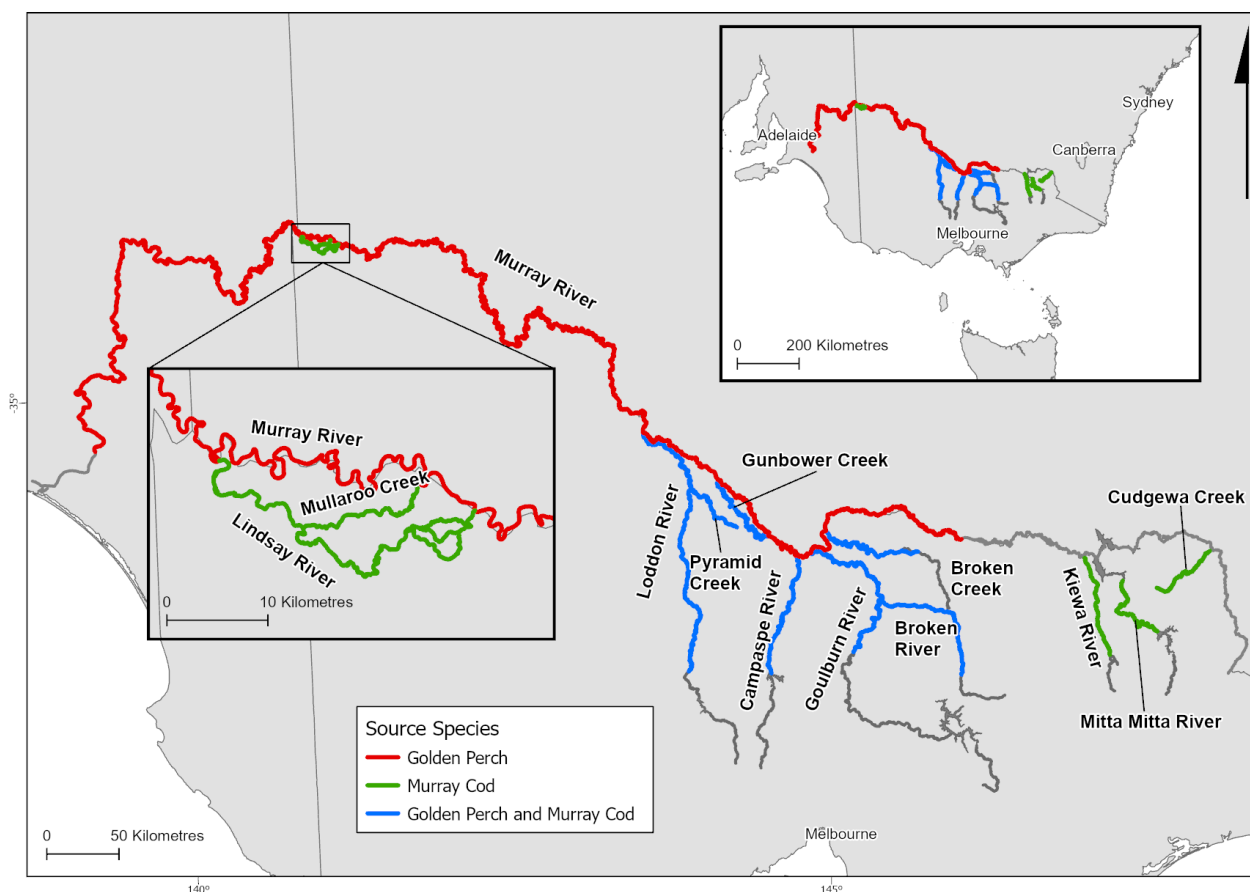


**Figure 1. Map of northern Victoria showing fish collection locations for the natal origin assessment.**

**Table 1. Summary of Murray Cod sample sizes for each waterway assigned to each cohort (birth year).**

| Location           | Birth Year |            |           |            |           |           |          |          |           |           |             |
|--------------------|------------|------------|-----------|------------|-----------|-----------|----------|----------|-----------|-----------|-------------|
|                    | ≤ 2015     | 2016       | 2017      | 2018       | 2019      | 2020      | 2021     | 2022     | 2023      | 2024      | Grand Total |
| Broken Creek       | 28*        | 6*         | 6*        | 3*         | 1*        |           | 1        | 1        |           | 2         | 48          |
| Broken River       |            | 33         | 4         | 22         | 6         |           |          |          | 4         | 14        | 83          |
| Campaspe River     |            | 7          | 4         | 12         | 2         | 4         |          | 3        | 9         | 16        | 57          |
| Cudgewa Creek      |            |            |           |            |           |           |          |          | 5         |           | 5           |
| Goulburn River     |            | 5          | 6         | 20         | 6         | 11        |          |          | 6         | 17        | 71          |
| Gunbower Creek     |            | 11         | 4         | 8          | 2         |           |          |          | 10        | 12        | 47          |
| Kiewa River        |            |            |           | 2          | 9         |           |          |          | 1         |           | 12          |
| Lindsay River      |            |            |           |            |           | 1         |          |          |           |           | 1           |
| Loddon River       |            |            |           |            |           |           |          | 2        | 8         | 6         | 16          |
| Mitta Mitta River  |            | 16         | 7         |            |           | 17        |          |          |           |           | 40          |
| Mullaroo Creek     |            |            |           | 9          |           | 7         |          |          |           |           | 16          |
| Pyramid Creek      |            |            |           | 2          | 9         |           |          | 1        | 2         | 5         | 19          |
| Hatcheries         |            | 30         | 17        | 64         | 22        | 12        |          |          |           | 3         | 148         |
| <b>Grand Total</b> | <b>28</b>  | <b>108</b> | <b>48</b> | <b>142</b> | <b>57</b> | <b>52</b> | <b>0</b> | <b>8</b> | <b>45</b> | <b>75</b> | <b>563</b>  |

\* Adult fish collected during fish death event

**Table 2. Summary of 2024/25 Golden Perch samples collected in Victorian tributaries and the Murray River used in the analysis.**

Collections of Golden Perch from 2018 (n=240) reported in Zampatti et al. (2019) not included.

| Species      | River          | Reach | No. fish   | Length (mm) |
|--------------|----------------|-------|------------|-------------|
| Golden Perch | Broken Creek   | 2-3   | 11         | 160 - 552   |
|              | Broken River   | 1-3   | 19         | 322 - 495   |
|              | Campaspe River | 2-4   | 33         | 164 - 528   |
|              | Goulburn River | 4-5   | 52         | 235 - 556   |
|              | Gunbower Creek | -     | 17         | 365 - 560   |
|              | Loddon River   | 4-5   | 10         | 109 - 540   |
|              | Murray River   | 1     | 47         | 260 - 539   |
|              | Murray River   | 2     | 34         | 292 - 527   |
|              | Murray River   | 3     | 51         | 164 - 510   |
|              | Murray River   | 4     | 56         | 100 - 537   |
|              | Pyramid Creek  | -     | 11         | 213 - 546   |
| <b>TOTAL</b> |                |       | <b>341</b> |             |

## 1.3 Analysis

### Juvenile Murray Cod

Otoliths collected from 371 juvenile Murray Cod (<180 mm TL, age <2 years), captured across 12 waterways, were assessed for  $^{87}\text{Sr}/^{86}\text{Sr}$  at the core and edge of otolith transverse sections, thereby representing the fish's natal origin and capture, respectively (see Tonkin et al. 2023). We also compared  $^{87}\text{Sr}/^{86}\text{Sr}$  of the samples from wild-caught fish with the otolith core values generated from 148 Murray Cod fingerlings sourced from the nine main hatcheries used by the VFA to stock the study waterways from 2016-2025. Samples of fingerlings were not available for two (new) hatcheries used in 2024-25. For these hatcheries, water samples were obtained from source water used to hatch eggs and rear larvae, with a  $^{87}\text{Sr}/^{86}\text{Sr}$  signature obtained for each sample as per methods described in Zampatti et al. (2019).

The age of each fish was determined by macroscopic age analysis of each otolith section, as described in Anderson et al. (1992). We then used microchemistry LA-ICPMS analysis of  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios from the otolith edge (from wild fish) and core (hatchery and wild fish) using an experimental system consisting of a 'Nu Plasma' multi-collector ICPMS (Nu Instruments, Wrexham, UK), coupled to a 'RESOLUTION' 193-nm excimer laser ablation system (Applied Spectra, USA). Each otolith was ablated on the primordium (core) and the dorsal margin at the widest radius using a 60- $\mu\text{m}$  laser spot. Ablation was performed under a pure helium (He) atmosphere followed by rapid transport of the ablated products to the MC-ICPMS in the argon carrier gas. Methods for online correction for isobaric interferences and mass bias are described in Woodhead et al. (2005), while accuracy and reproducibility was checked after every 10 otolith ablations using methods described in Thiem et al. (2022). The age of each fish was used to assign year cohorts (see Table 1 for sample sizes per cohort and waterway).

Otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  values were used to infer natal origin of juvenile Murray Cod by comparing the core (birth signature) to signatures representing (i) the waterway of capture (otolith edge  $^{87}\text{Sr}/^{86}\text{Sr}$  values) and (ii) the hatchery (otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$  values) used to stock fish in that waterway for a specific cohort (see Figure 2). First, waterway and hatchery signatures were tested for statistical distinctness. Where multiple hatcheries were used, each hatchery and cohort-specific waterway pairing was assessed. Natal origin for each fish collected (except those from the Broken Creek fish kill) was then evaluated using a simulation-based 'best-match' approach, comparing the otolith core value of each juvenile Murray Cod captured from the 12 waterways to (i) the distribution of otolith edge  $^{87}\text{Sr}/^{86}\text{Sr}$  values from wild-caught fish in the relevant

waterway, and (ii) the distribution of otolith core values from the relevant hatchery (see Appendix Table A11 and Table Appendix Table A2). For each fish, repeated resampling was used to estimate the probability that the otolith core value was more closely aligned with the waterway (wild) signature than the hatchery signature, from which a best-match metric (0-1.0) was derived.

Natal origin was assigned only where the best-match metric was  $\geq 0.95$  and the hatchery-waterway pairing associated with the best-matching comparison was statistically distinct (Tukey post-hoc  $P < 0.05$ ). Where a hatchery was not statistically distinct from the receiving waterway signature, individuals were assigned as 'Unknown (indistinct)' origin to minimise the risk of misclassifying stocked fish as wild-origin (or vice versa). Further to this, where multiple hatcheries were used for a waterway in a year, and one of those hatcheries was not statistically distinct from the waterway, fish that matched the waterway signature were also classified as 'Unknown (indistinct)', given they could be either wild or of the indistinct hatchery origin, while only those fish that matched statistically distinct hatchery values were assigned as stocked origin. Finally, for some waterways and years, information on the hatchery used was not available, or an individual fish was not matched to a hatchery used but also did not meet the  $\geq 0.95$  threshold for assignment. In such circumstances, individuals were classified as 'Unknown (hatchery unknown)' or 'Unknown ( $\geq 0.95$  best match)', respectively. All Murray Cod collected from Mullaroo Creek were classified wild-origin as fish were not stocked into this waterway between 2016-2025 (but were in the adjoining Lindsay River).

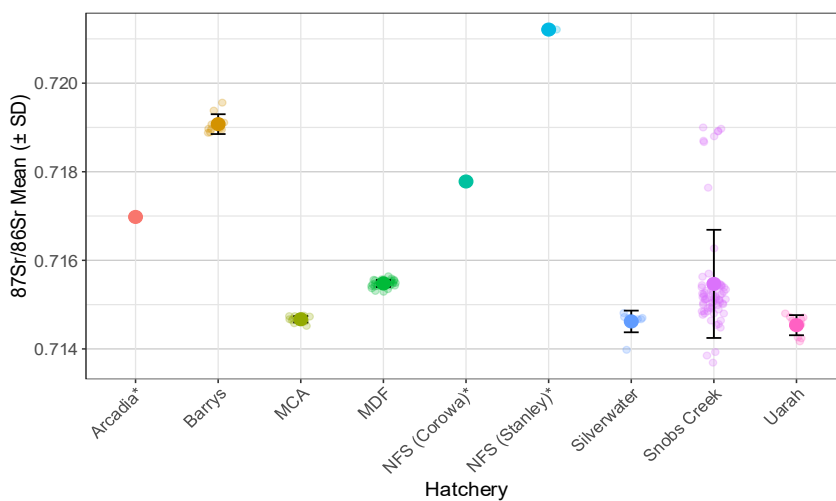


**Figure 2. Mean ( $\pm$ SD)  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures of hatcheries used to stock Murray Cod into northern Victorian waterways over the study period (all years combined).**

Raw values of individual fish or water samples (Arcadia and NFS hatcheries) presented in pale points.

**Table 3. Summary of natal assignment criteria used for juvenile Murray Cod.**

| Natal assignment                     | Criteria                                                                                                                                                                                                                                                                                                                                                                              |
|--------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Wild</b>                          | Otolith core $^{87}\text{Sr}/^{86}\text{Sr}$ best-match metric $\geq 0.95$ with the receiving waterway and the hatchery-waterway pairing associated with the best-matching comparison was statistically distinct (Tukey post hoc $P < 0.05$ ). Where multiple hatcheries were used in a cohort, wild assignment was only made if no indistinct hatchery-waterway pairing was present. |
| <b>Hatchery</b>                      | Otolith core $^{87}\text{Sr}/^{86}\text{Sr}$ best-match metric $\geq 0.95$ with a hatchery signature and the hatchery-waterway pairing for that hatchery was statistically distinct (Tukey post hoc $P < 0.05$ ).                                                                                                                                                                     |
| <b>Unknown (indistinct)</b>          | Hatchery-waterway pairing associated with the best-matching comparison (wild or hatchery) was not statistically distinct (Tukey post hoc $P > 0.05$ ), or at least one hatchery used within the cohort had an indistinct $^{87}\text{Sr}/^{86}\text{Sr}$ signature relative to the receiving waterway and the fish best matched the waterway (wild) signature.                        |
| <b>Unknown (hatchery unknown)</b>    | Hatchery reference signature(s) were unavailable for the waterway and cohort.                                                                                                                                                                                                                                                                                                         |
| <b>Unknown (&lt;0.95 best match)</b> | Hatchery-waterway pairing was statistically distinct, but the otolith core $^{87}\text{Sr}/^{86}\text{Sr}$ best-match metric did not meet the $\geq 0.95$ ( $p < 0.05$ ) threshold for assignment to either hatchery or waterway.                                                                                                                                                     |

## Golden Perch and adult Murray Cod

Analyses were conducted at the Advanced Geochemical Facility for Indian Ocean Research, University of Western Australia, using a Thermo Scientific NEPTUNE Plus multicollector ICP-MS coupled with a Teledyne Analyte G2 excimer laser ablation system. Otolith mounts were placed in the sample cell, and the primordium of each otolith was visually identified under a x400 objective with video imaging. The ablation path for each otolith sample was digitally plotted, with laser ablation settings and calibration following the methods described in (Zampatti et al. 2022).

As part of a previous study, a Bayesian Mixing Isotope Model (BMIM) was developed to map water  $^{87}\text{Sr}/^{86}\text{Sr}$  ratios across rivers in the Murray–Darling Basin (MDB) (Zampatti et al. 2019). The BMIM was developed from water samples collected at sites across the southern MDB from 2011–2021 and used a moving average structure similar to the spatial stream network model of Brennan et al. (2016), incorporating river branching. Although temporally static, the BMIM accounts for temporal variation through its residual error structure (Zampatti et al. 2019). An assignment algorithm was also developed to determine natal origin by comparing stationary sections of the otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  profile with river-specific mean values (and variance) and the distance from the capture location. The section closest to the otolith core represents the fish's natal origin (Zampatti et al. 2019).

Natal assignment was based on a Bayesian assignment framework (Brennan et al. 2019; Brennan and Schindler 2017) developed specifically for the major rivers within the MDB (Zampatti et al. 2019). In short, the algorithm looks for transitions between stationary periods in the  $^{87}\text{Sr}/^{86}\text{Sr}$  profiles (identified by regression trees), assigning probable locations backwards through time based on the previous location (starting from the capture location) and current  $^{87}\text{Sr}/^{86}\text{Sr}$  levels (using Bayes' Rule; Brennan et al. 2019; Zampatti et al. 2019). For the latter component, a MDB isoscape (map of  $^{87}\text{Sr}/^{86}\text{Sr}$  levels) was created by Zampatti et al. (2019). This isoscape includes mean ( $\mu_{\text{river}}$ ) and variation ( $\sigma_{\text{river}}$ ) for every major river in the MDB. Previous location affects the probability of new locations based on minimal river distance between river segments (e.g. touching river segments have distance of 0 km). An exception to the river distance penalty occurs during 200–800  $\mu\text{m}$  distance window of the  $^{87}\text{Sr}/^{86}\text{Sr}$  profile, in which a hatchery location is added as a possible option with no distant cost.

Following the initial algorithm run, two researchers reviewed all profiles to detect any obvious issues. Two key issues were identified: 1) the original 200–800  $\mu\text{m}$  hatchery band window missed clear hatchery signatures so we extended the range to 200–1100  $\mu\text{m}$ ; and 2) the Goulburn and Broken rivers couldn't be distinguished and were hence grouped together. These modifications were included in the second iteration.

Using the assignment matrix, we linked in region classification used in Zampatti et al. (2019). These classifications are based on spatial grouping, partly based on similar  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures and natural

spatial boundaries. For each fish, probabilities were summed for each river segment. Next, we extracted the natal origin segment and assigned each region one of the following categories: recruit (natal region same as capture region), stocked (natal origin is hatchery), and migrant (non-stocked fish in which natal region differs from capture region). The final natal origin status of either recruit/stocked/migrant was based on the most likely location (summed across all possibilities). With these definitions, there are two things to make clear. First, a recruit could have moved in and out of the capture region at some point. Second, a stocked fish might have been released in another region, and it migrated to the capture region.

## 2 Results

### 2.1 Murray Cod

#### *Juvenile Murray Cod*

Natal origin assignments based on otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$  values indicated that the relative contributions of natural recruitment and hatchery supplementation to Murray Cod populations varied substantially among waterways and cohorts across northern Victorian rivers (**Error! Reference source not found.**). Across the study, 20% of hatchery and cohort-specific waterway pairings had indistinct  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures (see Appendix Table A1 and Table Appendix Table A2). The lack of distinctness between hatchery and waterway pairings reduced the number of assignable fish in several waterways and cohorts, particularly in recent years as the number of hatcheries used increased. Overall, natal origin assignment was constrained in many waterways and cohorts by  $^{87}\text{Sr}/^{86}\text{Sr}$  value overlap between hatchery and waterway signatures and/or fish-level assignment uncertainty. These details are presented in Figure 3, with key results as follows:

- Wild recruits were most common in the Goulburn and Broken rivers for the 2016/17 and 2017/18 cohorts (generally 75–100% of fish in each cohort), and the Murrumbidgee River (100% of fish, acknowledging direct stocking only occurs in the adjacent Lindsay River). In recent years the certainty of this pattern in the Goulburn and Broken rivers is reduced due to increased use of hatcheries that do not have isotopically distinct water sources. For such recent cohorts (e.g. the Goulburn River 2024/25 cohort), fish whose otolith core signatures best matched the waterway could not be confidently assigned as wild. As such, the apparent absence of wild recruits in these cohorts should be interpreted cautiously, as it likely reflects assignment constraints rather than a true shift in recruitment dynamics. Moreover, based on previous years' assignments, it is likely this dominance of wild recruited fish would persist.
- Gunbower Creek continued to display pronounced cohort-to-cohort variability. Early cohorts (2016/17 and 2017/18) were dominated by hatchery-origin fish (approximately 75-90%; Figure 3), while the 2018/19 and 2024/25 cohorts comprised a substantial wild-origin component (approximately 85-90%).
- The Campaspe River natal assignments were less certain given the overlap in  $^{87}\text{Sr}/^{86}\text{Sr}$  values with several hatcheries or despite not matching to the hatchery did not meet the significance for matching to the waterway (i.e.  $p > 0.05$ ). This limited confident assignment in some cohorts and contributed to elevated unknown classifications. A similar result of natal origin assignment being of unknown classification occurred in multiple smaller waterways with small sample sizes.
- All Broken Creek cohorts were classified as one of the unknown categories, noting a very small sample size and two of the three cohorts sampled occurred in 2022/23 or after a fish kill, which was subject to significant stocking (recovery action). Similarly, the restricted cohorts from Cudgewa Creek (2023/24), Lindsay River (2020/21), Loddon River (2022–2024), and all Pyramid Creek cohorts (2018–2025) were entirely classified as unknown (except the Loddon River 2023/24 cohort which was 88%). Conversely, the most recent (2024/25) Loddon River cohort was assigned 85% stocked recruits.
- Data from the Pyramid Creek samples showed evidence of entirely wild origin in 2018/19 and 2019/20, with a significant match to the waterway signature and no stocking occurring in these years. Other years (which were subject to stocking) were either of unknown origin due to indistinct signatures or an unknown hatchery, however, otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$  values tightly clustered around the system edge signature (Figure 4). The median absolute difference between core and system edge values was 0.00011, with 75% of individuals within 0.000175 of the system median. Most core values fell within the interquartile range of the system edge distribution, indicating strong overlap with local water chemistry and, therefore, a high likelihood of being a wild recruited fish. One individual exhibited a larger deviation from the system median; however, the overall pattern was dominated by close correspondence between core and edge signatures suggesting either wild origin or alignment with a hatchery of the same signature (unknown at this stage).
- In the Kiewa River, mixed origin was observed in the 2019/20 cohort, with hatchery-origin fish comprising approximately 65% of assignable individuals and wild-origin fish comprising approximately 10%, while other cohorts remained dominated by unknown classifications (did not meet the significance level whilst also not matching to any hatchery). For the Mitta Mitta River, all

assignable fish from the 2016/17 and 2017/18 cohorts were hatchery-origin, while the 2020/21 cohort showed a higher contribution of wild-origin individuals (65%).

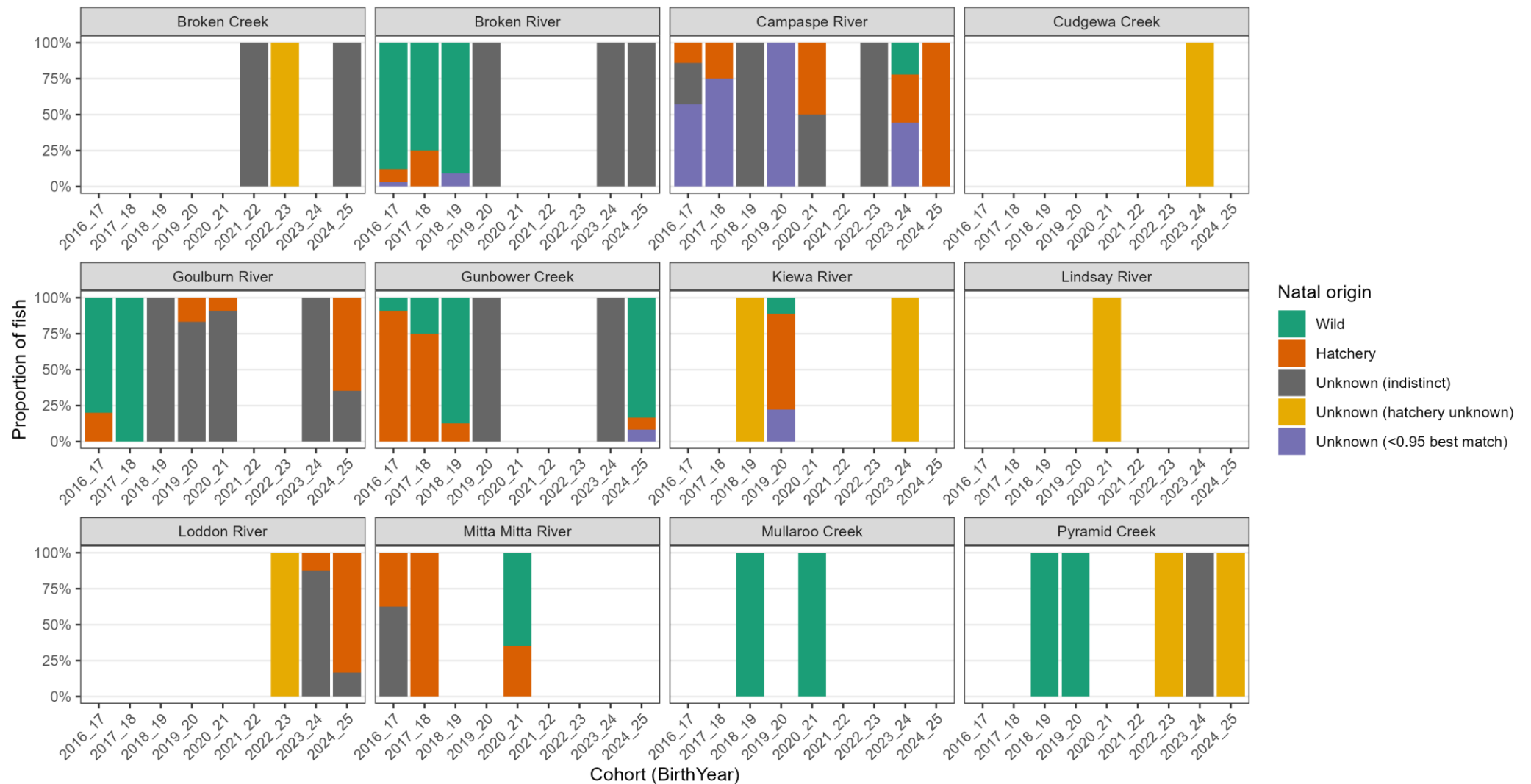


**Figure 3. Murray Cod natal origin assignments across study waterways and birth years, based on otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$  values.**

Assignments to wild or hatchery origin were made only where best-match confidence was significant ( $p < 0.05$ ) and the associated hatchery–waterway pairing was isotopically distinct. Fish associated with indistinct hatchery–waterway pairings, lacking hatchery reference data, or failing to meet the confidence threshold were classified as unknown.

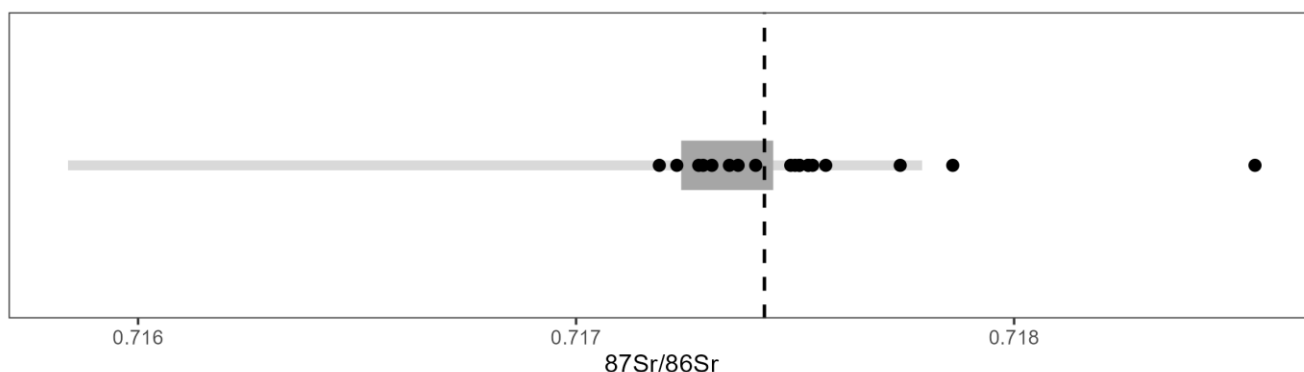


**Figure 4. Pyramid Creek: otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$  values relative to the system edge signature.**

Thick grey bar represents the interquartile range (IQR) of system edge  $^{87}\text{Sr}/^{86}\text{Sr}$  values; thin grey line indicates the full observed range. Points represent otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$  values for all individual Murray Cod collected from the Pyramid Creek. The dashed line shows the median system edge value.

#### *Broken Creek adult Murray Cod*

The adult sample of fish salvaged from the 2022 spring fish death event in Broken Creek was dominated by wild-origin individuals across a broad range of cohorts. Approximately 90% of adult Murray Cod were interpreted as wild-origin, with hatchery-origin individuals comprising a relatively small proportion of the sample (approximately 5%) and two (n=2) fish classified as migrant (

Table 4). The presence of wild adult fish spanning multiple age classes indicates that natural recruitment has contributed substantially to the Murray Cod population in Broken Creek over an extended period.

**Table 4. Age and natal origin assignment of adult Murray Cod salvaged from the 2022 spring fish death event in Broken Creek.**

| Age | Recruit % (n) | Migrant % (n) | Stocked % (n) |
|-----|---------------|---------------|---------------|
| 2   | 2.3 (1)       | 0             | 0             |
| 3   | 6.8 (3)       | 0             | 0             |
| 4   | 15.9 (7)      | 0             | 0             |
| 5   | 13.6 (6)      | 0             | 0             |
| 6   | 0             | 2.3 (1)       | 0             |
| 7   | 2.3 (1)       | 0             | 0             |
| 8   | 4.5 (2)       | 0             | 0             |
| 9   | 15.9 (7)      | 0             | 0             |
| 10  | 4.5 (2)       | 0             | 0             |
| 12  | 2.3 (1)       | 0             | 0             |
| 13  | 2.3 (1)       | 0             | 0             |
| 16  | 2.3 (1)       | 0             | 2.3 (1)       |
| 17  | 11.4 (5)      | 2.3 (1)       | 2.3 (1)       |

|    |         |   |   |
|----|---------|---|---|
| 18 | 4.5 (2) | 0 | 0 |
| 22 | 2.3 (1) | 0 | 0 |

## 2.2 Golden Perch

The Golden Perch samples from northern Victorian waterways and the Murray River in 2024-25 showed that natural recruits contributed approximately 62% of the sample, with stocked fish making up 38% (Figure 5). Of those naturally recruited fish, most were of a Murray River natal origin (~50% from the lower, mid and upper Murray combined), followed by the lower Darling (24%) and Goulburn-Broken (11%) rivers (Figure 5; Figure 6). A comparison with samples collected in 2018 (for those systems sampled) revealed similar compositions, albeit a slightly lower contribution of stocked fish (38% and 44% for 2024-25 and 2018, respectively) and higher contribution of migrant fish in 2024-25 (Table 5). Nevertheless, for specific year classes, this contribution along with that of migrant fish (fish recruited in other reaches), varied substantially across waterways as well as within waterways. Such nuances are presented in Figure 6, with key results as follows:

- The lower and mid Murray were the only reaches where stocked fish contributed <10% of the sample (Figure 6), with most fish being of lower Murray, lower Darling and mid Murray origin (Figure 5). For the upper Murray reach, the highest proportion of wild recruits were of Goulburn Broken (21%), upper Murray (11%) and mid Murray (6%) natal origin (Figure 5).
- The Goulburn River had similar contributions of fish compared to the 2018 assessment (Table 5) with 8%, 24% and 68% contribution of in-situ recruits, migrants and stocked fish, respectively (Figure 6). While having a low proportion of wild fish recruited in-situ, the Goulburn River contributed significant numbers of recruits to the adjoining Broken Creek (36%), upper Murray (21%) and lower Campaspe rivers (9%).
- The Campaspe River had a high contribution of stocked fish (76%), however migrant fish of mid Murray and Goulburn River origin contributed 18% of fish in the sample. The age structure of Golden Perch in the Campaspe River also reflected younger fish in comparison to some other rivers (particularly the Murray and Goulburn rivers), with most fish being <10 years of age (Figure 6) and most migrant fish being three years of age.
- While stocking contributed a high proportion of fish in the Gunbower Creek (41%), the same proportion of fish were migrants from a range of origins, including the Murrumbidgee, Loddon-Pyramid and Lower Darling rivers (Figure 5; Figure 5). We note the contribution of recruits (18%) could not be differentiated from mid Murray origin fish using the isoscape (due to the system being an anabranch of the mid-Murray). Thus, this value represents one, or a combination of either, source.
- The Broken River sample consisted entirely of fish ≥4 years of age, with most fish being captured upstream of major barriers and of stocked origin. Several fish were profiled as migrant fish of upper Murray origin, however, we suspect these have been incorrectly assigned. They are more likely stocked origin from a hatchery of an unknown Sr profile given 1. rapid natal transition along the otolith transect distance of 1000µm (usually typifies stocked fish – see methods; Figure 7); 2. barriers in the system and 3. The small size at which migration supposedly occurred.
- The Broken Creek sample had a particularly young age structure, with most fish ≤4 years of age (Figure 6). Fish were assigned as similar proportions of migrants, recruits and stocked origin, albeit from a small sample size. Note that recruit assignments were a collective Goulburn-Broken natal origin, given similar <sup>87</sup>Sr/<sup>86</sup>Sr signatures, but values were most similar to Goulburn River reaches.
- The Loddon River samples consisted largely of stocked fish (80%), with some migrant fish from the mid Murray River and lower Darling River reaches (albeit from a small sample size). The adjoining Pyramid Creek sample had 55% stocked origin with the remaining a combination of migrants from a range of sources, and potentially of Loddon-Pyramid natal origin. The latter was also present in samples from the Gunbower Creek, lower Murray, Goulburn and Broken rivers, although issues with differentiating these from the mid Murray in the isoscape presents a great deal of uncertainty.

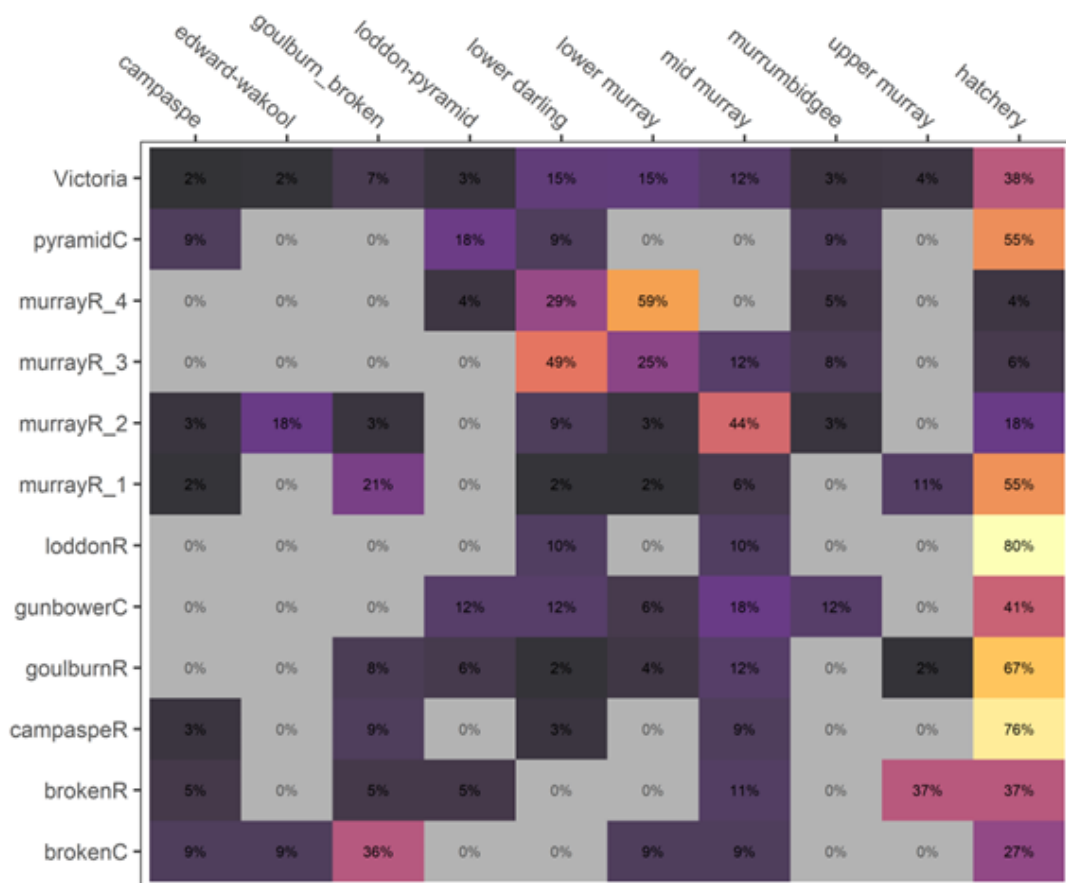


**Figure 5. Pairwise relationships for 2024-25 Golden Perch sample showing natal assignment proportions within each waterway.**

Rows show sampled waterways and columns the region of natal assignments (including hatchery origin).

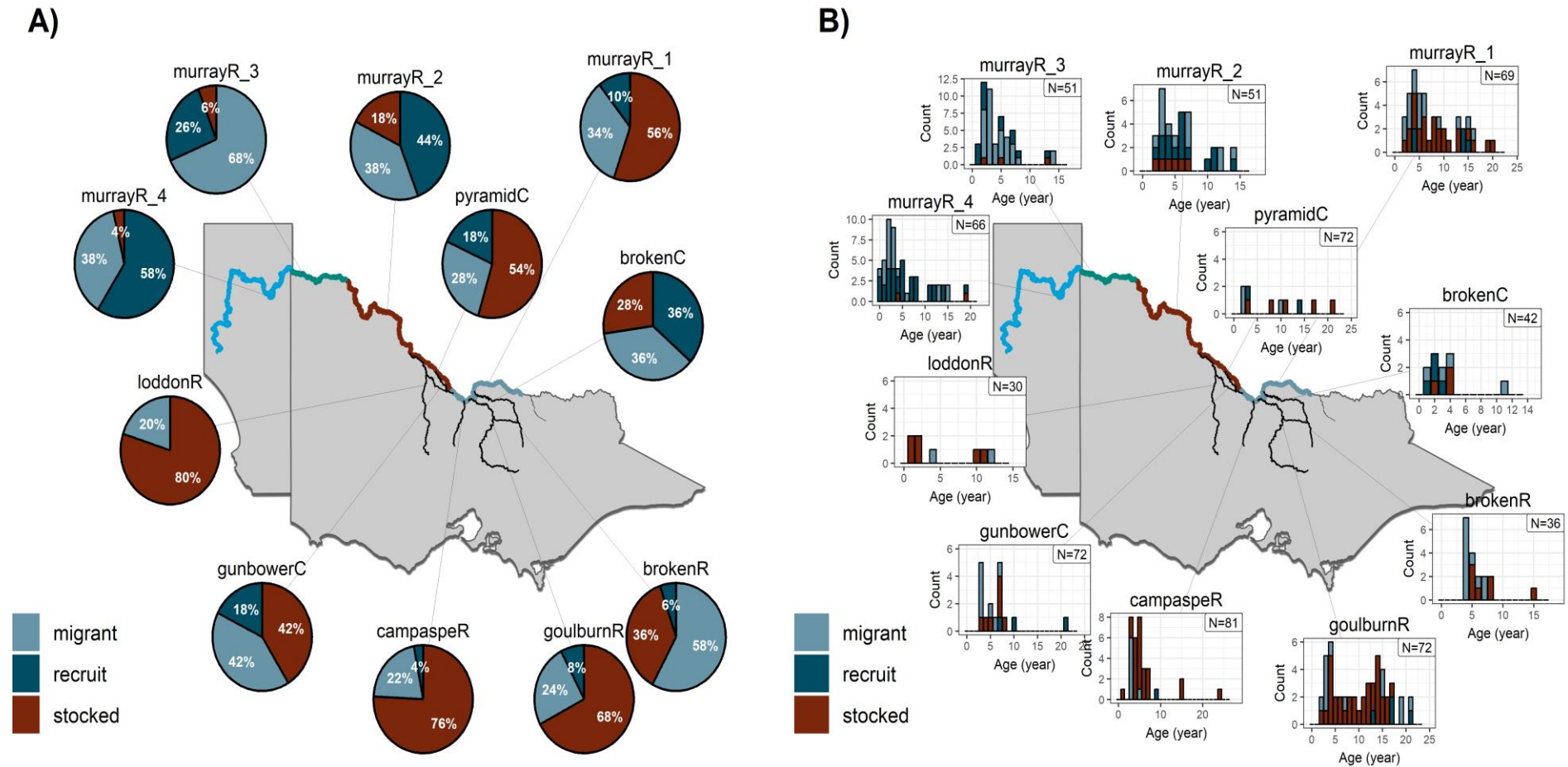


**Figure 6. Victoria-wide Golden Perch assignments for the 2024/25 sample.**

**Table 5. Summary of Golden Perch origin assessments for 2025 and 2018 across Victorian waterways and reaches of the Murray River.**

Sample sizes presented in parentheses.

| Waterway        | Year | Recruit       | Migrant       | Stocked      |
|-----------------|------|---------------|---------------|--------------|
| Broken Creek    | 2025 | 36.4%<br>(4)  | 36.4%<br>(4)  | 27.3%<br>(3) |
| Broken River    | 2025 | 5%<br>(1)     | 58%<br>(11)   | 37%<br>(7)   |
| Campaspe River  | 2018 | 0             | 4%<br>(1)     | 96%<br>(26)  |
|                 | 2025 | 3%<br>(1)     | 21%<br>(7)    | 76%<br>(25)  |
| Goulburn River  | 2018 | 7%<br>(3)     | 23%<br>(10)   | 70%<br>(30)  |
|                 | 2025 | 8%<br>(4)     | 25%<br>(13)   | 67%<br>(35)  |
| Gunbower Creek  | 2025 | 18%<br>(3)    | 41%<br>(7)    | 41%<br>(7)   |
| Loddon River    | 2018 | 0             | 0             | 100%<br>(5)  |
|                 | 2025 | 0             | 20%<br>(2)    | 80%<br>(8)   |
| Murray River R1 | 2018 | 9%<br>(4)     | 37%<br>(16)   | 53%<br>(23)  |
|                 | 2025 | 11%<br>(5)    | 34%<br>(16)   | 55%<br>(26)  |
| R2              | 2018 | 30%<br>(15)   | 52%<br>(26)   | 18%<br>(9)   |
|                 | 2025 | 44.1%<br>(15) | 38.2%<br>(13) | 17.6%<br>(6) |
| R3              | 2025 | 25%<br>(13)   | 69%<br>(35)   | 6%<br>(3)    |
|                 | 2018 | 37%<br>(21)   | 51%<br>(29)   | 12%<br>(7)   |
| R4              | 2025 | 59%<br>(33)   | 38%<br>(21)   | 4%<br>(2)    |
| Pyramid Creek   | 2018 | 13%<br>(2)    | 33%<br>(5)    | 53%<br>(8)   |
|                 | 2025 | 18.2%<br>(2)  | 27.3%<br>(3)  | 54.5%<br>(6) |

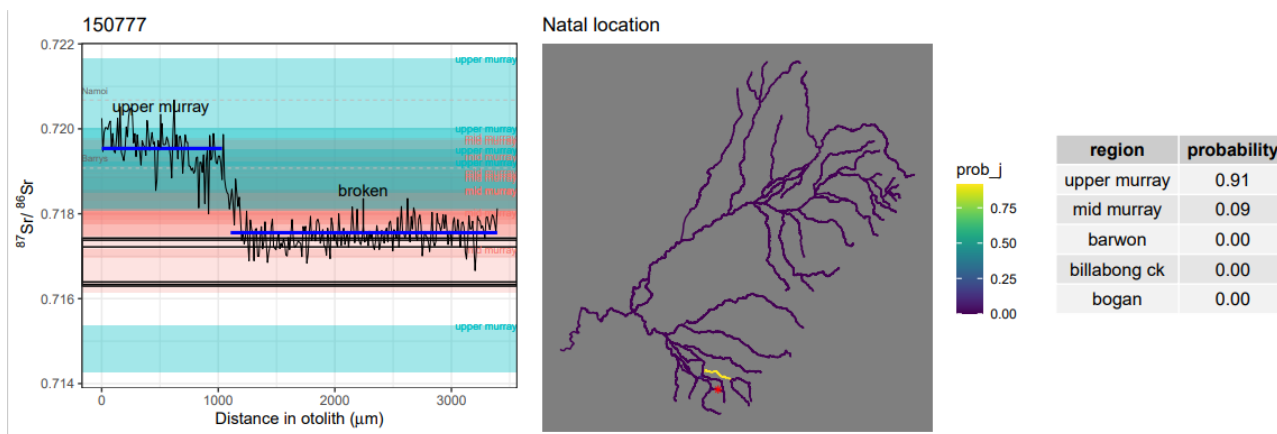


**Figure 7. Example of Broken River captured fish assigned as originating in the upper Murray River.**

Note fish is likely stocked from a hatchery of an unknown Sr profile given rapid natal transition around 1000 $\mu\text{m}$  (usually typifies stocked fish), barriers present and the small size at age the (upstream) migration supposedly occurred.

## 3 Discussion

### 3.1 Murray Cod

Otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  natal origin analysis revealed substantial spatial and temporal variability in the relative contributions of natural recruitment and hatchery supplementation to Murray Cod populations across northern Victoria. Strong evidence of wild recruitment was detected in specific systems and time periods. In particular, the Broken and Goulburn rivers showed high proportions of wild-origin fish in cohorts spanning 2016-2019, indicating periods of successful natural recruitment despite ongoing stocking. These results align with previous assessments of Murray Cod natal origin in the Goulburn River, which showed populations were largely comprised of wild-spawned fish (Tonkin et al. 2019). In more recent cohorts, confidence in wild recruitment in these systems was reduced due to increased use of hatcheries that could not be distinguished from the receiving waterways, limiting assignability of fish. Nevertheless, an increased proportion of fish assigned as hatchery origin for the 2024/25 cohort were present in the Goulburn River, with all fish matched to the Arcadia hatchery. The signature for this hatchery was derived from a single year of water signatures, which was similar to the Goulburn River value, thus should therefore be treated with some caution.

Waterways exhibiting mixed contributions of wild-origin and hatchery-origin Murray Cod, most notably the Gunbower Creek, Campaspe River and Pyramid Creek, illustrate the interplay between environmental conditions, water management and stocking in shaping population structure. In Gunbower Creek, pronounced cohort-to-cohort variability was evident, with early cohorts dominated by hatchery-origin fish and later cohorts showing an increasing contribution of wild recruits. This transition broadly coincides with periods of enhanced hydrological connectivity within and between Gunbower Creek, Gunbower Forest and the Murray River during large flow events and environmental watering, which are expected to improve habitat availability and conditions conducive to spawning and early survival (Stuart et al. 2019). The Campaspe River also exhibited mixed contributions, although a large portion of assignments were of unknown origin mainly due to a number of hatcheries used having  $^{87}\text{Sr}/^{86}\text{Sr}$  values indistinct to those of the Campaspe River. However, confirmation of wild recruits in some years, detection of natural spawning (VEFMAP unpublished data), and YoY collected from all sample reaches (Tonkin et al. 2025), some of which are not directly stocked, suggest wild recruits are likely contributing to at least some of those fish assigned as unknown origin. The Mitta Mitta River results indicated Murray Cod were predominantly of hatchery-origin, with mixed origins observed in the 2020/21 cohort only. This pattern is consistent with the expectation that recruitment success may be constrained in more heavily modified reaches and in waterways subject to additional stressors (e.g. altered thermal regimes and flow regulation) (Tonkin et al. 2023), which can affect spawning and early life-stage survival (Todd et al. 2005) as well as impact the survival of stocked fish (Tonkin et al. 2020).

Evidence from systems with limited or no hatchery reference data further supported the inferences drawn from otolith microchemistry. In Pyramid Creek, where all juvenile cohorts were classified as unknown due to indistinct or unavailable hatchery signatures, otolith core  $^{87}\text{Sr}/^{86}\text{Sr}$  values were tightly clustered around the system edge signature, particularly in the 2018/19 cohort. With most of these samples being from the 2018/19 year, which was not stocked, there is compelling support for local recruitment. Similarly, the dominance of wild-origin individuals across multiple age classes in adult Murray Cod salvaged from Broken Creek indicates that natural recruitment has contributed substantially to population persistence over extended timeframes (Jones et al. 2017). Recent juvenile cohorts, collected after the fish death event, however, remain difficult to resolve isotopically. Together, these lines of evidence reinforce the conclusion that natural recruitment plays a meaningful, system-specific role in sustaining Murray Cod populations across northern Victoria (and the Murray River). The detectability of natural recruitment using otolith microchemistry is however strongly mediated by hatchery practices, reference data availability and isotopic distinctness.

Interpretation of in-situ recruitment was strongly influenced by the treatment of hatchery-waterway combinations with indistinct signatures. Across several systems, particularly those with increasing numbers of hatcheries supplying fish in recent years, the presence of hatchery-waterway combinations with indistinct signatures substantially limited the proportion of individuals for which natal origin (particularly wild-origin) could be confidently assigned. This was most evident in recent cohorts in the Goulburn and Broken rivers, where overlap between hatchery and waterway  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures increased the prevalence of unknown classifications.

### 3.2 Golden Perch

Golden Perch populations in northern Victorian waterways and the Murray River during 2024–25 exhibited a mixed origin structure, with natural recruits comprising approximately 62% of the sample and stocked fish contributing 38%. This composition is broadly consistent with previous assessments (Zampatti et al. 2019), although the proportion of stocked fish has declined slightly (38% vs 44%) and migrant contributions have increased. These changes likely reflect several years of high flows and floods, generating large-scale recruitment events in the lower Murray and Darling rivers (e.g., 2021/22 cohort; Rees et al. 2023; Jones et al. 2023; Stuart et al. 2024) and subsequent dispersal opportunities past major barriers along the Murray River and Victorian tributaries (e.g. Stuart et al. 2024).

Like previous assessments, most naturally recruited fish in the sample originated from the Murray River (31% combined from lower, mid, and upper reaches), followed by the lower Darling (15%) and Goulburn–Broken systems (7%), with varying contribution of these systems across waterways. The lower Murray reaches (reaches 3 and 4) had minimal stocked fish (<10%), dominated instead by local recruits and migrants of lower Darling River origin. Nevertheless, the contribution of natural recruits declined dramatically in upstream reaches (above Lock 11), with a much smaller contribution of fish originating from the lower Murray and Darling rivers. The same pattern emerged in Victorian tributaries, with samples largely being dominated by stocked or more local recruitment sources. This pattern likely reflects the limited connectivity with downstream recruitment sources (from Lock 11; e.g. Jones et al. 2025b). Gunbower Creek displayed a more balanced structure, with stocked fish and migrants each contributing 42%, while recruits accounted for 18%; however, this estimate is confounded by its anabranch relationship with the mid-Murray.

For fish of non-stocked origin in upstream reaches of the Murray River, local recruitment sources, particularly the mid and upper Murray River and the Goulburn–Broken system, made a notable contribution. Unlike the lower Murray and Darling rivers, where age classes reflect large but episodic recruitment events, recruitment in the mid and upper Murray and Goulburn rivers appears relatively consistent across age classes, albeit at low levels. Spawning has been documented annually in the upper Murray River (King et al. 2016) and likely occurs in the mid Murray River (Rees et al. 2023), supported by the permanent lotic conditions of these reaches, which maintain flowing characteristics even under average discharge (Mallen-Cooper and Zampatti 2018). Although less consistent than the upper Murray River, the Goulburn River also provides conditions conducive to Golden Perch spawning, particularly since the implementation of environmental water deliveries to promote such events (Koster et al. 2017; Webb et al. 2022). However, recruitment outcomes from these interventions have been poorly documented. This has remained a point of contention regarding the value of flow delivery aimed at enhancing spawning outcomes without evidence of subsequent recruitment. These findings provide evidence of recruitment success and contribution to the broader population. The results also emphasize that assessing recruitment outcomes for migratory species, -especially pelagophilic fish such as Golden Perch, requires measures of subsequent year-class strength (rather than only young-of-year presence) as well as sampling beyond spawning locations. This is demonstrated by the Goulburn River results in our study, which contributed recruits to the upper Murray and adjoining tributaries. Indeed, similar results are emerging for Silver Perch in the mid – upper reaches of the Murray River (River Murray Channel monitoring program, unpublished data).

While we did not conduct any detailed assessment of age structure and subsequent drivers of cohort strength, there were some large differences in samples collected across the systems. The Campaspe River populations were skewed toward younger age classes (<10 years), with most migrants being three-year-olds, indicative of recent dispersal events. Similarly, Broken Creek's young age structure suggests recent colonization or stocking in the wake of the 2022 flood and fish death event (Stoios et al. 2023). These findings highlight the influence of episodic events such as floods, fish deaths, recruitment pulses and stocking programs on demographic composition (e.g. Thiem et al. 2020).

Our results reinforce that Golden Perch populations in Victorian tributaries remain heavily shaped by stocking and migration, with natural recruitment playing a secondary but variable role. For reaches well connected to the Murray River, management strategies should prioritize actions that enhance connectivity opportunities (e.g. Jones et al. 2025a). For reaches with shortfalls in connectivity to natal sources, and as such, a high stocking reliance (e.g., upper Campaspe and Loddon river reaches), stocking will need to be maintained. Indeed, these results should be used in the development, or revision, of waterway objectives (particularly those for environmental water) specifically targeting outcomes for Golden Perch. More specifically for Victorian tributaries, outside of the Goulburn River and perhaps Gunbower Creek, there is little evidence to support recruitment to the broader metapopulation. As such, and as has been occurring in recent FLOWS reviews and Seasonal Watering Plans, water management (under current volumes and constraints) targeting outcomes for Golden Perch in these tributaries is best targeted at supporting connectivity and providing cues to enhance immigration from other sources (such as the Murray River), as well as providing suitable baseflows to maintain suitable habitat for these and stocked individuals. Of course, the contribution of hatchery releases to population persistence must still be considered. Future work

should integrate genetic assessments and long-term demographic monitoring to evaluate implications for population resilience and genetic diversity (Harrisson et al. 2025).

### 3.3 Limitations

A key limitation for interpreting natal origin results was that assignments could not be made where waterway signatures were not clearly distinguishable from stocking hatchery signatures. This presents a major limitation of the otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  technique, if waterways are stocked with fish sourced from hatcheries which share similar chemical signatures. As such, if waterway managers and fisheries agencies do not coordinate efforts to use fish sourced from hatcheries of distinguishable origin for stocking programs, then the otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  technique is of little value. Other techniques, especially those that are non-lethal like genetics, exist but require marking of fish (Crook et al. 2007) or collection of parent material (e.g. Lutz et al. 2021). For species such as Murray Cod and Golden Perch, that are stocked widely and produced at many private hatcheries, however these techniques are often labour intensive and difficult to coordinate. Moreover, unless all fish are marked or parents sampled, such techniques still cannot provide conclusive results.

For the Golden Perch results specifically, a key assumption for assigning natal origin relies on the accuracy of the  $^{87}\text{Sr}/^{86}\text{Sr}$  isoscape (Zampatti et al. 2019). The isoscape used in this study was constructed from water samples across the MDB. The assignment model assumes that the Sr signature comes from one of the river segments in the isoscape. Thus, if fish are using other water habitats with different Sr signatures, misclassification will happen. Therefore, the spatial completeness of the isoscape is critical. Another source of variation will be temporal variation within a spatial location. Sampling of the isoscape varies in temporal coverage of flow conditions across the MDB. Coverage of temporal variation in the Southern MDB is low and hence this could be a source of misclassification. Low sampling coverage does not necessarily mean poor estimates, just the potential for higher variability than estimated. A final point is that the algorithm assumes the fish Sr signature has to come from the isoscape. The current algorithm iteration does not allow for 'unknown' locations whereby even if the assigned river does not fit the Sr profile, it is assigned to that river given all the other possibilities fit much worse.

The aggregation of individual fish results to produce a cohort or waterway level assignment was also constrained by low sample sizes (for some waterways or cohorts). Sample sizes were largely reliant on the presence and capture of fish during routine monitoring, with a general pattern of large sample sizes coinciding with strong natural recruitment events (as previously reported for the Broken and Goulburn rivers in Harris et al. 2020). Of course, the number of fish stocked (which is increasing in many Victorian waterways) may also influence the composition of a population natal source. Overall, these limitations highlight the need to interpret natal origin results alongside other population metrics, such as abundance, cohort composition, spatial distribution and population genetic composition. Genetic data, in particular, provides powerful insights into species' status and trends by revealing patterns of genetic diversity, population structure, and connectivity, inbreeding and relatedness (often a product of stocking). Indeed, this was recently presented for Golden Perch using earlier collections of otoliths (our 2018 sample) and genetic material (Harrisson et al. 2025).

### 3.4 Conclusions

This study demonstrates that natal origin and recruitment dynamics of Murray Cod and Golden Perch across northern Victorian waterways and the Murray River are highly variable in space and time, reflecting the combined influence of hydrology, connectivity, waterway modification and hatchery supplementation on population structure. Otolith microchemistry revealed strong evidence of natural recruitment in some systems and time periods, alongside mixed-origin and stocking-dominated populations in others. Although uncertainty in natal assignments persists where hatchery and waterway signatures overlap, explicitly accounting for indistinct hatchery-waterway pairings provides a conservative basis to interpret recruitment sources and avoid over-estimating wild/stocked recruitment where signatures cannot be resolved. Collectively, these findings reinforce the importance of combining natal origin information with demographic monitoring to understand population dynamics. They also underscore the value of strategically aligning environmental water delivery and stocking programs to support sustainable native fish populations.

From a management perspective, these results also provide critical context to interpret recruitment signals observed in routine fish population monitoring and to evaluate progress toward Basin Plan biological objectives related to fish population structure and natural recruitment. Further, increases in abundance or year-class strength cannot be interpreted in isolation, particularly in stocked or highly connected systems where changes in such metrics may reflect a combination of local spawning, stocking and migration. For Murray Cod, evidence of sustained or intermittent natural recruitment in some northern Victorian waterways

indicates that water management (including environmental water delivery) can support key life-history processes under suitable conditions, even in highly regulated waterways such as the Broken, Goulburn, and Campaspe rivers and Gunbower Creek. For Golden Perch, results reaffirm the dominant influence of large-scale recruitment events and longitudinal connectivity along the Murray River, with tributary populations reflecting varying dependence on migration and stocking, notwithstanding the contribution of recruits from the Goulburn system to broader population demographics. These results also provide a strong basis to develop or revise water-management objectives for Victorian tributaries, emphasising that—given limited local recruitment occurs in systems outside the Goulburn River—environmental water should focus on enhancing connectivity, facilitating immigration from the Murray River, and maintaining baseflows that sustain habitat for resident and stocked individuals.

Importantly, identifying waterways with mixed or emergent natural recruitment capacity highlights opportunities for targeted evaluation of flow outcomes and adaptive management. Incorporating natal origin data alongside year-class strength, age structure and abundance metrics will improve the ability of programs (such as state and federal environmental water monitoring programs) to attribute ecological responses to management actions. It will also reduce uncertainty in reporting against Basin Plan objectives, and guide more efficient use of environmental water and stocking to support resilient native fish populations. Indeed, further interrogation of these results is required to recognise when and where waterways retain the capacity for natural recruitment. This would provide an opportunity to strategically leverage environmental water to reduce long-term reliance on stocking, while maintaining supplementation where environmental constraints limit consistent recruitment.

Given the increasing number of fish being stocked, and number of hatcheries being used, differentiating fish between stocked and hatchery origin is becoming increasingly challenging. If a need to distinguish between wild and hatchery origin fish (for recruitment assessment purposes for example) remains, better communication and coordination between waterway managers and fisheries agencies is needed, with stocking for specific waterways using fish sourced from hatcheries of distinguishable origin. Without this approach, the otolith  $^{87}\text{Sr}/^{86}\text{Sr}$  technique is of limited value, and other measures such as genetics or marking should be adopted, acknowledging these still require extra effort and often more coordination. Of further value, interpreting results with other population metrics, such as abundance, cohort composition, spatial distribution (including stocking sites) and long-term population genetic composition will provide further insights into the drivers (such as river flows and number of fish released), and dynamics of recruitment and populations.

## References

- Anderson, J., Morison, A. and Ray, D. (1992). Age and growth of Murray cod, *Maccullochella peelii* (Perciformes: Percichthyidae), in the lower Murray-Darling Basin, Australia, from thin-sectioned otoliths. *Marine and Freshwater Research* **43**, 983–1013.
- Birnie-Gauvin, K., Costantini, D., Cooke, S.J. and Willmore, W.G. (2017). A comparative and evolutionary approach to oxidative stress in fish: a review. *Fish and Fisheries* **18**, 928–942.
- Brennan, S. R., Cline, T. J. and Schindler, D. E. (2019). Quantifying habitat use of migratory fish across riverscapes using space–time isotope models. *Methods in Ecology and Evolution* **10**, 1036–1047.
- Brennan, S. R. and Schindler, D. E. (2017). Linking otolith microchemistry and dendritic isoscapes to map heterogeneous production of fish across river basins. *Ecological Applications* **27**, 363–377.
- Cadwallader, P. L. (1978). Some causes of decline in range and abundance of native fish in the Murray–Darling River system. *Proceedings of the Royal Society of Victoria* **90**, 211–224.
- Cadwallader, P. L. (1986). A review of the decline of native fish in the Murray–Darling system and associated fishery management problems. *Australian Journal of Marine and Freshwater Research* **37**, 123–137.
- Crook, D. A., O'Mahony, D., Gillanders, B. M., Munro, A. R. and Sanger, A. C. (2007). Production of external fluorescent marks on Golden Perch fingerlings through osmotic induction marking with Alizarin Red S. *North American Journal of Fisheries Management* **27**, 670–675.
- Harris, A., Tonkin, Z., Moloney, P., and Woodhead, J. (2020) Using Otolith Microchemistry to Assign Natal Origin to Juvenile Murray Cod. Unpublished Client Report for Water and Catchments, Department of Environment, Land, Water and Planning. Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, Heidelberg, Victoria.
- Harrisson, K. A., Beheregaray, L. B., Bice, C. M., Booth, E. J., Brauer, C. J., Butler, G. L., Dawson, D., Dudchenko, O., Fanson, B. G., Hackett, G., Harris, A., Iscaro, B., Ingram, B., Kaur, P., Koster, W. M., Krusic-Golub, K., Lieschke, J., Lutz, M. L., Lyon, J., Mallen-Cooper, M., Morrongiello, J. R., Murphy, N. P., O'Dwyer, J., O'Connor, J., Pavlova, A., Pickworth, A., Rourke, M. L., Sadekov, A., Strawbridge, A., Sun, P., Thiem, J. D., Tonkin, Z., Wainwright, A., Wilson, P. J., Woods, R., Yen, J. D. L. and Zampatti, B. P. (2025). Otolith and genomic data reveal temporal insights into stocking across a large river basin in a mobile, long-lived Australian freshwater fish species. *Molecular Ecology* **34**, e17714.
- Jones, C., Durkin, L., Khwaja, N., O'Connor, J., Vivian, L., Cornell, G., Kitchingman, A. and Clunie, P. (2023). Victorian Murray Floodplain Restoration Project: Ecology baseline monitoring 2022/23 – Mallee CMA region. Arthur Rylah Institute for Environmental Research, Department of Energy, Environment and Climate Action, Heidelberg, Victoria.
- Jones, M. J., Moloney, P., Hale, R., Stuart, I., O'Connor, J. and Tonkin, Z. (2017). Lower Broken Creek fish population survey. Unpublished Client Report for the Goulburn Broken Catchment Management Authority. Arthur Rylah Institute for Environmental Research, Heidelberg, Victoria.
- Jones, M. J., Moloney, P., Hale, R., O'Connor, J., Vivian, L., Amtstaetter, F., Fanson, B., Taylor, M. D., Stuart, I., Baumgartner, L., Yee, J. and Tonkin, Z. (2025a). More than the sum of their parts—Environmental flows increase fish movement and fishway functionality. *Ecological Engineering* **214**, 107566.
- Jones, M. J., Iscaro, B. and Fanson, B. (2025b). Fish passage at the Mildura weir and navigation lock. Unpublished Client Report for the Commonwealth environmental Water Holder and Department of Energy, Environment and Climate Action. Arthur Rylah Institute for Environmental Research, Department of Energy, Environment and Climate Action, Heidelberg, Victoria.
- King, A.J., Tonkin, Z. and Mahoney, J. (2009) Environmental flow enhances native fish spawning and recruitment in the Murray River, Australia. *River Research and Applications* **25**, 1205–1218.
- King, A. J., Gwinn, D. C., Tonkin, Z., Mahoney, J., Raymond, S. and Beesley, L. (2016). Using abiotic drivers of fish spawning to inform environmental flow management. *Journal of Applied Ecology* **53**, 34–43.
- Koehn, J.D., King, A.J., Beesley, L., Copeland, C., Zampatti, B.P., and Mallen-Cooper, M. (2014). Flows for native fish in the Murray-Darling Basin: lessons and considerations for future management. *Ecological Management & Restoration* **15**, 40–50.

- Koster, W. M., Dawson, D. R., Liu, C., Moloney, P. D., Crook, D. A. and Thomson, J. R. (2017). Influence of streamflow on spawning-related movements of Golden Perch *Macquaria ambigua* in south-eastern Australia. *Journal of Fish Biology* **90**, 93–108.
- Lintermans, M. (2023). Fishes of the Murray-Darling Basin: an introductory guide. Canberra, Murray-Darling Basin Commission. Second edition. (Australian River Restoration Centre Limited) 288pp.
- Lutz, M. L., Tonkin, Z., Yen, J. D., Johnson, G., Ingram, B. A., Sharley, J., Lyon, J., Chapple, D. G., Sunnucks, P. and Pavlova, A. (2021). Using multiple sources during reintroduction of a locally extinct population benefits survival and reproduction of an endangered freshwater fish. *Evolutionary Applications* **14**, 950–964.
- Lyon, J. P., Bird, T., Tonkin, Z., Raymond, S., Sharley, J. and Hale, R. (2021). Does life history mediate discharge as a driver of multi-decadal changes in populations of freshwater fish? *Ecological Applications* **31**(8). e02430.
- Mallen-Cooper, M. and Zampatti, B. P. (2018). History, hydrology and hydraulics: rethinking the ecological management of large rivers. *Ecohydrology* **11**, e1965.
- Pebesma, E. (2018). Simple features for R: Standardized support for spatial vector data. *The R Journal* **10**, 439–446. <https://doi.org/10.32614/RJ-2018-009>
- R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.
- Rees, G. N., Zampatti, B., McInerney, P., Biswas, T. K., Bice, C. and Tonkin, Z. (2023). River Murray Channel Monitoring 2022–2023: a technical report to the Commonwealth Environmental Water Office. CSIRO Land & Water, Canberra, Australia.
- Stoios, R., Tonkin, Z., Yen, J., Kitchingman, A. and Lyon, J. (2023). Floodwater short-term impacts and potential recovery paths of freshwater fish populations. Unpublished report for the Department of Energy, Environment and Climate Action – Water and Catchments Division. Arthur Rylah Institute for Environmental Research, Department of Energy, Environment and Climate Action.
- Stuart, I., Fanson, B. and Thiem, J. (2024). Native fish movement in the Great Darling Anabranch 2022–23. Report for the Darling Anabranch Adaptive Management Monitoring Program (DAAMMP) and Commonwealth Environmental Water Holder.
- Stuart, I., Sharpe, C., Stanislawski, K., Parker, A. and Mallen-Cooper, M. (2019). From an irrigation system to an ecological asset: adding environmental flows establishes recovery of a threatened fish species. *Marine and Freshwater Research* **70**, 1295–1306.
- Thiem, J. D., Baumgartner, L. J., Fanson, B., Sadekov, A., Tonkin, Z. and Zampatti, B. P. (2022). Contrasting natal origin and movement history informs recovery pathways for three lowland river species following a mass fish kill. *Marine and Freshwater Research* **73**, 237–246.
- Thiem, J. D., Wooden, I. J., Baumgartner, L. J., Butler, G. L., Taylor, M. D. and Watts, R. J. (2020). Hypoxic conditions interrupt flood-response movements of three lowland river fish species: implications for flow restoration in modified landscapes. *Ecohydrology* **13**, e2197.
- Todd, C. R., Ryan, T., Nicol, S. J. and Bearlin, A. R. (2005). The impact of cold-water releases on the critical period of post-spawning survival and its implications for Murray Cod (*Maccullochella peelii peelii*): a case study of the Mitta Mitta River, south-eastern Australia. *River Research and Applications* **21**, 1035–1052.
- Tonkin, Z., Kitchingman, A., Ingram, B., Lieschke, J., Koster, W., Lyon, J., Lutz, M. and Pavlova, A. (2019). Smarter stocking: a synthesis of existing data to assess native fish stocking success in Victorian rivers. Unpublished Client Report for the Victorian Fisheries Authority. Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, Heidelberg, Victoria.
- Tonkin, Z., Jones, C., Clunie, P., Vivian, L., Amtstaetter, F., Jones, M., Koster, W., Mole, B., O'Connor, J. and Brooks, J. (2020). Victorian Environmental Flows Monitoring and Assessment Program: Stage 6 Synthesis Report 2016–2020. Arthur Rylah Institute for Environmental Research Technical Report Series No. 316. Department of Environment, Land, Water and Planning, Heidelberg, Victoria.
- Tonkin, Z., Koehn, J., Commens, S., Hackett, G., Harris, A., Kitchingman, A., Lyon, J., Moloney, P., Todd, C. and Woodhead, J. (2023). Using multiple lines of evidence to assess recovery potential of a warm-water fish population in a cold-water-impacted river. *Frontiers in Conservation Science* **4**, 16.

- Tonkin, Z., Yen, J., Kitchingman, A., Amtstaetter, F., Hackett, G., Harris, A., Koster, W., Lieschke, J., Lyon, J., Pickworth, A. and Raymond, S. (2024). Assessing long-term population trends for priority fish species across Victorian rivers. Arthur Rylah Institute for Environmental Research Unpublished Report. Department of Energy, Environment and Climate Action, Heidelberg, Victoria.
- Tonkin, Z., Dawson, D., Harris, A., Hackett, G., Kitchingman, A. and Yen, J. (2025). Monitoring fish populations in northern Victorian rivers 2025. Arthur Rylah Institute for Environmental Research Unpublished Report. Department of Energy, Environment and Climate Action, Heidelberg, Victoria.
- Webb, J. A., Guo, D., Koster, W. M., Lauchlan-Arrowsmith, C. and Vietz, G. J. (2022). Can hydraulic measures of river conditions improve our ability to predict ecological responses to changing flows? Flow velocity and spawning of an iconic native Australian fish. *Frontiers in Environmental Science* **10**.
- Woodhead, J., Swearer, S., Hergt, J. and Maas, R. (2005). In situ Sr-isotope analysis of carbonates by LA-MC-ICP-MS: interference corrections, high spatial resolution and an example from otolith studies. *Journal of Analytical Atomic Spectrometry* **20**, 22–27.
- Zampatti, B., Fanson, B., Strawbridge, A., Tonkin, Z., Thiem, J., Butler, G., Balcombe, S., Brooks, S., Woods, R., Koster, W., Lyon, J., King, A., Crook, D., Baumgartner, L. and Doyle, K. (2019). Basin-scale population dynamics of Golden Perch and Murray Cod: relating flow to provenance, movement and recruitment in the Murray–Darling Basin. In: Price, A., Balcombe, S., Humphries, P., King, A. and Zampatti, B. (Eds). Murray–Darling Basin Environmental Water Knowledge and Research Project Fish Theme Research Report. Report prepared for the Department of the Environment and Energy, Commonwealth Environmental Water Office, Albury–Wodonga: La Trobe University.
- Zampatti, B. P., Fanson, B. G., Baumgartner, L. J., Butler, G. L., Brooks, S. G., Crook, D. A., Doyle, K., King, A. J., Koster, W. M., Maas, R., Sadekov, A., Scott, P., Strawbridge, A., Thiem, J. D., Tonkin, Z., Wilson, P. J., Woodhead, J. and Woods, R. (2022). Population demographics of Golden Perch (*Macquaria ambigua*) in the Darling River prior to a major fish kill: a guide for rehabilitation. *Marine and Freshwater Research* **73**, 223–236.

## Appendix

**Appendix Table A1. Hatchery-Waterway pairings assigned as ‘unknown’ origin in the conservative framework natal origin analysis**

**Note:** These cohorts were assigned as ‘unknown’ origin due to a lack of statistical distinctness between stocking hatchery and receiving waterway signatures (Tukey post-hoc;  $P > 0.05$ ) unless additional distinct hatcheries were used in the same year and identified.

| Hatchery     | Stocking Year (Cohort) | Waterway          | Tukey Post-Hoc Results       |
|--------------|------------------------|-------------------|------------------------------|
| Arcadia      | 2023/24                | Goulburn River    | F1,5=0.009366123, P=0.9267   |
| Arcadia      | 2024/25                | Broken Creek      | F1,1=0.7095648, P=0.5543     |
| Barrys       | 2016/17                | Mitta Mitta River | F1,24=0.6850357, P=0.416     |
| MDF          | 2016/17                | Campaspe River    | F1,15=0.4050188, P=0.5341    |
| MDF          | 2018/19                | Campaspe River    | F1,20=0.7096823, P=0.4095    |
| NFS (Corowa) | 2023/24                | Broken River      | F1,3=0.4381809, P=0.5553     |
| NFS (Corowa) | 2024/25                | Broken River      | F1,13=2.671087, P=0.1262     |
| Snobs Creek  | 2018/19                | Goulburn River    | F1,43=0.06664885, P=0.7975   |
| Snobs Creek  | 2019/20                | Broken River      | F1,29=0.0882267, P=0.7686    |
| Snobs Creek  | 2019/20                | Goulburn River    | F1,29=0.001509108, P=0.9693  |
| Snobs Creek  | 2019/20                | Gunbower Creek    | F1,25=1.687699, P=0.2058     |
| Snobs Creek  | 2020/21                | Campaspe River    | F1,27=0.4470956, P=0.5094    |
| Snobs Creek  | 2020/21                | Goulburn River    | F1,34=1.929357, P=0.1739     |
| Snobs Creek  | 2021/22                | Broken Creek      | F1,24=0.2569235, P=0.6169    |
| Snobs Creek  | 2023/24                | Broken River      | F1,27=0.8904456, P=0.3537    |
| Snobs Creek  | 2023/24                | Loddon River      | F1,31=0.2613145, P=0.6128    |
| Snobs Creek  | 2023/24                | Pyramid Creek     | F1,25=5.108116e-07, P=0.9994 |
| Snobs Creek  | 2024/25                | Goulburn River    | F1,40=0.05355787, P=0.8182   |
| Snobs Creek  | 2024/25                | Loddon River      | F1,29=0.4578951, P=0.504     |

**Appendix Table A2. Mean ( $\pm$  SD)  $^{87}\text{Sr}/^{86}\text{Sr}$  signatures for waterways and stocking hatcheries by cohort.**

**Note:** Symbols indicate Tukey post-hoc outcomes for hatchery–waterway comparisons (\* distinct, ns indistinct, – untestable, < 3 samples and/or hatchery unknown).

| Location       |               | Cohort                      |                             |                             |                              |                             |                              |                           |                              |                             |
|----------------|---------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|-----------------------------|------------------------------|---------------------------|------------------------------|-----------------------------|
| System         | Hatchery      | 2016_17                     | 2017_18                     | 2018_19                     | 2019_20                      | 2020_21                     | 2021_22                      | 2022_23                   | 2023_24                      | 2024_25                     |
| Broken Creek   |               |                             |                             |                             |                              |                             | 0.7171 (n=1)                 | 0.7158 (n=1)              |                              | 0.7177 $\pm$ 0.0007 (n=2)   |
| Broken Creek   | Barrys        |                             |                             |                             |                              |                             | 0.7191 $\pm$ 0.0002 (n=10)*  |                           | 0.7191 $\pm$ 0.0002 (n=10)*  |                             |
| Broken Creek   | Snobs Creek   |                             |                             |                             |                              |                             | 0.7162 $\pm$ 0.0018 (n=25)ns |                           |                              |                             |
| Broken Creek   | Arcadia       |                             |                             |                             |                              |                             |                              |                           | 0.7170 (n=1)-                | 0.7170 (n=1)-               |
| Broken Creek   | NFS (Corowa)  |                             |                             |                             |                              |                             |                              |                           | 0.7178 (n=1)-                |                             |
| Broken Creek   | NFS (Stanley) |                             |                             |                             |                              |                             |                              |                           | 0.7212 (n=1)-                |                             |
| Broken River   |               | 0.7178 $\pm$ 0.0007 (n=33)  | 0.7174 $\pm$ 0.0003 (n=3)   | 0.7171 $\pm$ 0.0004 (n=22)  | 0.7160 $\pm$ 0.0011 (n=6)    |                             |                              |                           | 0.7171 $\pm$ 0.0009 (n=4)    | 0.7174 $\pm$ 0.0002 (n=14)  |
| Broken River   | Snobs Creek   | 0.7150 $\pm$ 0.0004 (n=30)* | 0.7151 $\pm$ 0.0004 (n=17)* | 0.7162 $\pm$ 0.0018 (n=25)* | 0.7162 $\pm$ 0.0018 (n=25)ns | 0.7162 $\pm$ 0.0018 (n=25)- | 0.7162 $\pm$ 0.0018 (n=25)-  |                           | 0.7162 $\pm$ 0.0018 (n=25)ns | 0.7162 $\pm$ 0.0018 (n=25)* |
| Broken River   | Silverwater   |                             | 0.7146 $\pm$ 0.0002 (n=9)*  |                             |                              |                             |                              |                           |                              |                             |
| Broken River   | Barrys        |                             |                             |                             |                              | 0.7191 $\pm$ 0.0002 (n=10)- | 0.7191 $\pm$ 0.0002 (n=10)-  |                           |                              |                             |
| Broken River   | Arcadia       |                             |                             |                             |                              |                             |                              | 0.7170 (n=1)-             |                              |                             |
| Broken River   | NFS (Corowa)  |                             |                             |                             |                              |                             |                              | 0.7178 (n=1)-             | 0.7178 (n=1)-                | 0.7178 (n=1)ns              |
| Broken River   | NFS (Stanley) |                             |                             |                             |                              |                             |                              | 0.7212 (n=1)-             | 0.7212 (n=1)-                | 0.7212 (n=1)*               |
| Campaspe River |               | 0.7155 $\pm$ 0.0004 (n=7)   | 0.7154 $\pm$ 0.0007 (n=4)   | 0.7156 $\pm$ 0.0001 (n=12)  | 0.7150 $\pm$ 0.0005 (n=2)    | 0.7169 $\pm$ 0.0019 (n=4)   |                              | 0.7158 $\pm$ 0.0002 (n=3) | 0.7159 $\pm$ 0.0001 (n=9)    | 0.7157 $\pm$ 0.0000 (n=16)  |

| Location       |               | Cohort                     |                         |                           |                           |                           |                          |               |                        |                           |
|----------------|---------------|----------------------------|-------------------------|---------------------------|---------------------------|---------------------------|--------------------------|---------------|------------------------|---------------------------|
| System         | Hatchery      | 2016_17                    | 2017_18                 | 2018_19                   | 2019_20                   | 2020_21                   | 2021_22                  | 2022_23       | 2023_24                | 2024_25                   |
| Campaspe River | MDF           | 0.7155±0.000<br>1 (n=10)ns |                         | 0.7155±0.0001<br>(n=10)ns | 0.7155±0.0001<br>(n=22)*  |                           |                          |               |                        |                           |
| Campaspe River | Snobs Creek   | 0.7150±0.000<br>4 (n=30)*  |                         |                           |                           | 0.7162±0.0018<br>(n=25)ns | 0.7162±0.0018<br>(n=25)- |               |                        |                           |
| Campaspe River | Silverwater   |                            | 0.7146±0.0002<br>(n=9)* |                           |                           |                           |                          |               |                        |                           |
| Campaspe River | Barrys        |                            |                         |                           |                           | 0.7191±0.0002<br>(n=10)*  | 0.7191±0.0002<br>(n=10)- |               |                        |                           |
| Campaspe River | NFS (Corowa)  |                            |                         |                           |                           |                           |                          | 0.7178 (n=1)- |                        | 0.7178 (n=1)*             |
| Campaspe River | NFS (Stanley) |                            |                         |                           |                           |                           |                          | 0.7212 (n=1)- |                        | 0.7212 (n=1)*             |
| Campaspe River | Arcadia       |                            |                         |                           |                           |                           |                          |               | 0.7170 (n=1)*          | 0.7170 (n=1)*             |
| Goulburn River |               | 0.7168±0.000<br>2 (n=5)    | 0.7168±0.0003<br>(n=6)  | 0.7161±0.0002<br>(n=20)   | 0.7163±0.0003<br>(n=6)    | 0.7170±0.0001<br>(n=11)   |                          |               | 0.7170±0.0006<br>(n=6) | 0.7161±0.0001<br>(n=17)   |
| Goulburn River | Barrys        | 0.7191±0.000<br>2 (n=10)*  |                         |                           |                           |                           |                          |               |                        |                           |
| Goulburn River | Snobs Creek   | 0.7150±0.000<br>4 (n=30)*  |                         | 0.7162±0.0018<br>(n=25)ns | 0.7162±0.0018<br>(n=25)ns | 0.7162±0.0018<br>(n=25)ns | 0.7162±0.0018<br>(n=25)- |               |                        | 0.7162±0.0018<br>(n=25)ns |
| Goulburn River | Silverwater   |                            | 0.7146±0.0002<br>(n=9)* |                           |                           |                           |                          |               |                        |                           |
| Goulburn River | MDF           |                            |                         |                           | 0.7155±0.0001<br>(n=22)*  |                           |                          |               |                        |                           |
| Goulburn River | MCA           |                            |                         |                           |                           | 0.7147±0.0001<br>(n=12)*  |                          |               |                        |                           |
| Goulburn River | Uarah         |                            |                         |                           |                           |                           | 0.7145±0.0002<br>(n=10)- |               |                        |                           |
| Goulburn River | Arcadi        |                            |                         |                           |                           |                           |                          | 0.7170 (n=1)- | 0.7170 (n=1)ns         | 0.7170 (n=1)*             |
| Goulburn River | NFS (Corowa)  |                            |                         |                           |                           |                           |                          |               |                        | 0.7178 (n=1)*             |

| Location          |               | Cohort                |                       |                       |                        |                       |                       |                     |                        |                        |
|-------------------|---------------|-----------------------|-----------------------|-----------------------|------------------------|-----------------------|-----------------------|---------------------|------------------------|------------------------|
| System            | Hatchery      | 2016_17               | 2017_18               | 2018_19               | 2019_20                | 2020_21               | 2021_22               | 2022_23             | 2023_24                | 2024_25                |
| Goulburn River    | NFS (Stanley) |                       |                       |                       |                        |                       |                       |                     |                        | 0.7212 (n=1)*          |
| Gunbower Creek    |               | 0.7186±0.0007 (n=11)  | 0.7180±0.0001 (n=4)   | 0.7181±0.0001 (n=8)   | 0.7179±0.0000 (n=2)    |                       |                       |                     | 0.7181±0.0001 (n=10)   | 0.7181±0.0001 (n=12)   |
| Gunbower Creek    | MDF           | 0.7155±0.0001 (n=10)* |                       |                       |                        |                       |                       |                     |                        |                        |
| Gunbower Creek    | Snobs Creek   | 0.7150±0.0004 (n=30)* | 0.7151±0.0004 (n=17)* | 0.7162±0.0018 (n=25)* | 0.7162±0.0018 (n=25)ns |                       |                       |                     |                        | 0.7162±0.0018 (n=25)*  |
| Gunbower Creek    | Uarah         |                       |                       |                       |                        | 0.7145±0.0002 (n=10)- |                       |                     |                        |                        |
| Gunbower Creek    | MCA           |                       |                       |                       |                        |                       | 0.7147±0.0001 (n=12)- |                     |                        |                        |
| Gunbower Creek    | NFS (Corowa)  |                       |                       |                       |                        |                       |                       | §0.7178 (n=1)-      |                        |                        |
| Gunbower Creek    | NFS (Stanley) |                       |                       |                       |                        |                       |                       | §0.7212 (n=1)-      |                        |                        |
| Gunbower Creek    | Arcadia       |                       |                       |                       |                        |                       |                       |                     | §0.7170 (n=1)-         |                        |
| Kiewa River       |               |                       |                       | 0.7158±0.0002 (n=2)   | 0.7153±0.0003 (n=9)    |                       |                       |                     |                        | 0.7166 (n=1)           |
| Kiewa River       | Silverwater   |                       |                       |                       | 0.7146±0.0002 (n=9)*   |                       |                       |                     |                        |                        |
| Loddon River      |               |                       |                       |                       |                        |                       |                       | 0.7146±0.0020 (n=2) | 0.7159±0.0001 (n=8)    | 0.7167±0.0001 (n=6)    |
| Loddon River      | Arcadia       |                       |                       |                       |                        |                       |                       |                     | §0.7170 (n=1)*         | 0.7170 (n=1)*          |
| Loddon River      | MCA           |                       |                       |                       |                        |                       |                       |                     | 0.7147±0.0001 (n=12)*  | -                      |
| Loddon River      | Snobs Creek   |                       |                       |                       |                        |                       |                       |                     | 0.7162±0.0018 (n=25)ns | 0.7162±0.0018 (n=25)ns |
| Mitta Mitta River |               | 0.7189±0.0007 (n=16)  | 0.7183±0.0003 (n=7)   |                       |                        | 0.7186±0.0002 (n=17)  |                       |                     |                        |                        |

| Location          |               | Cohort                     |                          |                           |                         |                          |                          |                          |                           |                        |
|-------------------|---------------|----------------------------|--------------------------|---------------------------|-------------------------|--------------------------|--------------------------|--------------------------|---------------------------|------------------------|
| System            | Hatchery      | 2016_17                    | 2017_18                  | 2018_19                   | 2019_20                 | 2020_21                  | 2021_22                  | 2022_23                  | 2023_24                   | 2024_25                |
| Mitta Mitta River | Barrys        | 0.7191±0.000<br>2 (n=10)ns |                          |                           |                         |                          |                          |                          |                           |                        |
| Mitta Mitta River | MDF           | 0.7155±0.000<br>1 (n=10)*  |                          |                           |                         |                          |                          |                          |                           |                        |
| Mitta Mitta River | Snobs Creek   |                            | 0.7151±0.0004<br>(n=17)* | 0.7162±0.0018<br>(n=25)ns |                         |                          |                          |                          |                           |                        |
| Mitta Mitta River | Silverwater   |                            |                          |                           | 0.7146±0.0002<br>(n=9)* |                          |                          |                          |                           |                        |
| Mitta Mitta River | MCA           |                            |                          |                           |                         | 0.7147±0.0001<br>(n=12)* | 0.7147±0.0001<br>(n=12)- | 0.7147±0.0001<br>(n=12)- |                           |                        |
| Mitta Mitta River | NFS (Corowa)  |                            |                          |                           |                         |                          |                          | §0.7178 (n=1)-           | §0.7178 (n=1)-            | 0.7178 (n=1)-          |
| Mitta Mitta River | NFS (Stanley) |                            |                          |                           |                         |                          |                          | §0.7212 (n=1)-           | §0.7212 (n=1)-            | 0.7212 (n=1)-          |
| Pyramid Creek     |               |                            |                          | 0.7177±0.0001<br>(n=2)    | 0.7174±0.0000<br>(n=9)  |                          |                          | 0.7158 (n=1)             | 0.7162±0.0002<br>(n=2)    | 0.7173±0.0001<br>(n=5) |
| Pyramid Creek     | Snobs Creek   |                            |                          |                           |                         |                          |                          |                          | 0.7162±0.0018<br>(n=25)ns |                        |

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