*Molecular Ecology*

WILEY

MOLECULAR ECOLOGY

ORIGINAL ARTICLE OPEN ACCESS

# Otolith and Genomic Data Reveal Temporal Insights Into Stocking Across a Large River Basin in a Mobile, Long-Lived Australian Freshwater Fish Species

Katherine A. Harrisson[1,2,3] | Luciano B. Beheregaray[4] | Christopher M. Bice[5,6] | Emily J. Booth[4] | Chris J. Brauer[4] | Gavin L. Butler[7] | David Dawson[3] | Olga Dudchenko[8,9] | Benjamin G. Fanson[3] | Graeme Hackett[3] | Annique Harris[3] | Matthew J. Jones[3] | Parwinder Kaur[10] | Wayne M. Koster[3] | Kyne Krusic-Golub[11] | Erez Lieberman Aiden[8,9] | Jason A. Lieschke[3] | Jarod Lyon[3] | John R. Morrongiello[12] | Nicholas P. Murphy[1,2] | James O'Dwyer[1,2] | Scott M. C. Raymond[3] | Meaghan L. Rourke[13] | Arron Strawbridge[5] | Jason D. Thiem[13] | Zeb Tonkin[3] | Jian D. L. Yen[3] | Brenton P. Zampatti[14]

[1]Department of Environment and Genetics, La Trobe University, Melbourne, Australia | [2]Research Centre for Future Landscapes, La Trobe University, Melbourne, Australia | [3]Department of Energy, Environment and Climate Action, Arthur Rylah Institute for Environmental Research, Melbourne, Victoria, Australia | [4]Molecular Ecology Laboratory, College of Science and Engineering, Flinders University, Adelaide, South Australia, Australia | [5]South Australian Research and Development Institute (SARDI), Aquatic Sciences, South Australia, Australia | [6]School of Biological Sciences, The University of Adelaide, Adelaide, South Australia, Australia | [7]Department of Primary Industries and Regional Development, Grafton Fisheries Centre, Grafton, New South Wales, Australia | [8]Department of Molecular and Human Genetics, The Center for Genome Architecture, Baylor College of Medicine, Houston, Texas, USA | [9]The Center for Theoretical Biological Physics, Rice University, Houston, Texas, USA | [10]UWA School of Agriculture and Environment, University of Western Australia, Perth, Australia | [11]Fish Ageing Services Pty Ltd., Queenscliff, Victoria, Australia | [12]School of BioSciences, University of Melbourne, Melbourne, Australia | [13]Department of Primary Industries and Regional Development, Narrandera Fisheries Centre, Narrandera, New South Wales, Australia | [14]Commonwealth Scientific and Industrial Research Organisation (CSIRO), Environment Reseach Unit, Glen Osmond, South Australia, Australia

**Correspondence:** Katherine A. Harrisson (k.harrisson@latrobe.edu.au)

**Received:** 30 September 2024 | **Revised:** 15 February 2025 | **Accepted:** 19 February 2025

**Handling Editor:** J. A. H. Benzie

**Funding:** This work was supported by Australian Research Council (DE190100636, LP0667952, FT130101068 and DP190102533), La Trobe University, The University of Melbourne, Goulburn Broken Catchment Management Authority, US-Israel Binational Science Foundation Award (2019276), NIH Encyclopedia of DNA Elements Mapping Center Award (UM1HG009375), Welch Foundation (Q-1866), National Human Genome Research Institute of the National Institutes of Health (RM1HG011016-01A1), Behavioral Plasticity Research Institute (NSF DBI-2021795), NSF Physics Frontiers Center Award (NSF PHY-2019745).

**Keywords:** conservation genetics | conservation management | fish | fisheries genomics | fisheries management | wildlife management

## ABSTRACT

Freshwater ecosystems and their biota are under increasing pressure from anthropogenic stressors. In response to declining fish stocks, hatchery and stocking programmes are widely implemented as core components of restoration and management strategies, with positive outcomes for some wild populations. Despite this, stocking remains contentious due to potential genetic and ecological risks to wild populations. Monitoring and evaluation of stocking outcomes are critical to ensuring the long-term sustainability of wild populations, but identification of stocked individuals post-release remains a key challenge, particularly for mobile species. In this study, we combined otolith (natal origin and age) and genomic data to identify stocked individuals and evaluate the genetic implications of stocking for a culturally and socioeconomically important and mobile freshwater fish, golden perch *Macquaria ambigua* (family: Percichthyidae), across Australia's Murray–Darling Basin (MDB). We also generated

a chromosome-level genome assembly. Many close kin were detected across the MDB, increasing in prevalence over recent decades and mostly of hatchery origin. Rivers with many close kin were associated with low effective population sizes ($N_e < 100$). Genetic signatures of stocking varied according to local context, being most pronounced in but not restricted to rivers considered functionally isolated for management purposes. Where fish are stocked into rivers that are part of the connected metapopulation, there is scope to modify current stocking practices to avoid over-representation of related stocked individuals. Increased focus on the genetic diversity of stocked fish is likely to promote the long-term persistence of golden perch in the wild.

## 1 | Introduction

Freshwater ecosystems and their fish fauna are under immense threat from anthropogenic stressors (Flitcroft et al. 2019). Climate change, habitat loss and fragmentation, altered flow regimes, pollution, introduced fishes and overharvesting have caused considerable declines in the abundance of many fish species (Reid et al. 2019). In response, management agencies often implement large-scale releases of hatchery-reared fry or juvenile fish into the wild (stocking, hereafter) as core components of their restoration and management strategies and to support recreational or commercial fisheries (Cowx 1994; Welcomme and Bartley 1998; Hunt and Jones 2018). Despite its wide use in fisheries management, stocking remains contentious owing to the potential genetic and ecological risks it can pose to wild fish populations (Hunt and Jones 2018; Claussen and Philipp 2023; McMillan et al. 2023; Radinger et al. 2023).

Understanding the potential effects of stocking on wild fish populations is complex, as these effects vary across species and hatchery programmes and occur via a range of interacting pathways (e.g., ecological or genetic processes, disease, fishing; Naish et al. 2007; Araki and Schmid 2010; McMillan et al. 2023). Stocking using the offspring of wild-caught broodstock as part of conservation recovery-type efforts can be an effective management tool for the demographic and genetic restoration of depleted wild populations (Ferchaud et al. 2018; Janowitz-Koch et al. 2019; Lutz et al. 2021; Buckley et al. 2024; Pavlova et al. 2024) and for reintroductions (Lyon et al. 2012; Liermann et al. 2017; Marshall et al. 2022). However, adverse effects of larger-scale releases of hatchery-origin fish on the genetic diversity, productivity and abundance of wild populations are common in many traditional production and supplementation hatchery programmes targeted primarily at boosting commercial or recreational fisheries (Araki and Schmid 2010; McMillan et al. 2023).

Disruption to genetic processes is one of the most reported ways in which wild fish populations are adversely impacted by stocking (Araki and Schmid 2010; McMillan et al. 2023). Hatchery-origin individuals are highly susceptible to rapid genetic changes (e.g., adaptation to the hatchery environment, inbreeding and outbreeding depression, founder effects, genetic drift) and are often reported as having lower fitness compared to wild individuals (Thériault et al. 2011; O'Sullivan et al. 2021; Bouchard et al. 2022; Shedd et al. 2022). In some cases, natural selection may act to purge maladaptive alleles introduced via stocking and to maintain local adaptations in wild populations (e.g., Janowitz-Koch et al. 2019). In other cases, stocking with maladaptive genotypes may adversely affect the fitness of wild populations (Araki et al. 2007). Where stocking is based on large numbers of individuals from insufficient founders, it can lead to genetic swamping of wild gene pools with close kin and subsequent reductions in effective population size (Ryman and Laikre 1991; Christie et al. 2012; Gossieaux et al. 2019; Klütsch et al. 2021). Effective population size is distinct from census population size and is an important estimator in wildlife conservation and management because it is intrinsically linked to key evolutionary processes (including natural selection) that influence the capacity of populations to maintain genetic diversity and adapt to environmental change (Frankham et al. 2014). Therefore, in the absence of informed genetic management, stocking has the potential to compromise the adaptive capacity and long-term persistence of wild populations (Hare et al. 2011; Frankham et al. 2019), even in cases where short-term demographic boosts are observed initially (May et al. 2024).

Given the potential ecological and genetic risks, monitoring and critical evaluation of stocking are essential to ensuring the long-term sustainability of wild fish populations (Claussen and Philipp 2023). However, the evaluation of the effects of stocking on wild populations is relatively uncommon and challenging, in part due to the difficulties involved in identifying stocked individuals once they have been released into the wild (Crook et al. 2009). Methods involving physical tagging or adipose fin clipping of stocked individuals are impractical for species that are stocked at juvenile life stages and in very large numbers (Uglem et al. 2020). Genetic signatures of population structure are commonly used to identify stocked individuals in some fish species, particularly those characterised by high natal philopatry and strong population structure, such as salmonids (Utter 2004). However, these approaches are more challenging for species characterised by lower levels of population genetic structure, such as those with high effective population sizes and/or high dispersal capacity, including those with a migratory life history (Bootsma et al. 2021). Genetic parentage and kinship methods (e.g., parentage-based tagging, whereby stocked individuals are identified via assignment to databases of broodstock parents) have been widely used for decades in salmonids but can be logistically challenging to implement at large spatial scales (Steele et al. 2019). The use of natural or artificial chemical tags incorporated into fish otoliths (ear bones) represents an alternative method for discrimination of hatchery origin but one that requires lethal sampling (e.g., Curtis et al. 2014; Reis-Santos et al. 2023). Otolith information alone also does not provide information about the potential population-level implications of stocking, including the genetic contributions of stocked fish to recruitment in the wild and effects on genetic diversity and adaptive potential in wild populations.

In the Murray–Darling Basin (MDB) in Australia, stocking is a management strategy widely used to recover threatened native freshwater fish species and support recreational fishing of depleted populations. Fisheries management in the MDB is particularly complex, as it covers over 1 million km$^2$ of semi-arid

landscapes and encompasses the jurisdictional boundaries of five states and territories. One of the most heavily stocked native freshwater fish species in the MDB is the golden perch *Macquaria ambigua* (family: Percichthyidae). The golden perch ('Dhagaay' in Gamilaraay/Kamilaroi and Yuwaalaraay languages and 'Gagalin' or 'Bidyin' in Wiradjuri language) is culturally important to First Nations people and, as a popular angling species, is of significant socioeconomic value.

The golden perch is a migratory species with a high dispersal capacity across life stages, which has led to high gene flow and limited population genetic structure in the MDB (Faulks et al. 2010; Attard et al. 2018, 2022; Stuart and Sharpe 2020; Zampatti et al. 2021; Booth et al. 2024). Despite existing as a relatively well-connected metapopulation across much of the MDB, golden perch is susceptible to flow regulation and the presence of artificial barriers (e.g., dams/weirs) that restrict fish movement (Zampatti 2019). As a result, local population size and degree of interconnectedness vary spatially across the MDB, and golden perch populations in many reaches are considered locally depauperate, functionally disconnected and reliant on stocking for local persistence (Table 1; Zampatti et al. 2019; Butler et al. 2024).

Since the 1970s, tens of millions of golden perch have been stocked into the MDB from government and private hatcheries to support recreational fisheries, with rates of stocking increasing through time (Hunt and Jones 2018; Table 1). Although many stocked golden perch are released into lakes, impoundments or disconnected parts of river systems with depauperate wild populations (e.g., upstream of major artificial barriers such as dams or weirs), they are also increasingly stocked into areas where they have potential to interact with the wild metapopulation (Hunt and Jones 2018; Table 1). To minimise impacts on wild populations, hatchery guidelines for golden perch typically include the use of natural-origin

**TABLE 1** | Stocking records: Number of golden perch stocked into sampled river reaches during different time periods. Also indicated is whether sampled reaches comprise largely areas that are considered part of the connected metapopulation (M) or are considered for management purposes largely functionally disconnected by barriers and reliant on stocking for local persistence (FD).

| Code | Reach | Con | 1990–1999 | 2000–2009 | 2010–2019 | Total (1990–2019) |
|---|---|---|---|---|---|---|
| LMU | Lower Murray | M | 0 | 0 | 0 | 0 |
| MUL | Mullaroo-Lindsay | M | 0 | 0 | 0 | 0 |
| LDA | Lower Darling (below Menindee) | M | 0 | 35,000 | 16,000 | 51,000 |
| UDA | Upper Darling (above Menindee) | M | 0 | 9000 | 58,130 | 67,130 |
| WIM | Wimmera | FD | 359,550 | 380,050 | 634,800 | 1,374,400 |
| MAQ | Macquarie | FD | 16,200 | 331,502 | 232,793 | 580,495 |
| GIN | Gingham | FD | 0 | 0 | 0 | 0 |
| GWY | Gwydir | FD | 86,997 | 239,844 | 72,288 | 399,129 |
| MAC | Macintyre | M | 62,700 | 165,341 | 132,295 | 360,336 |
| SEV | Severn | M | 39,600 | 95,742 | 49,672 | 185,014 |
| DUM | Dumaresq | FD | 0 | 7600 | 25,636 | 33,236 |
| MBG | Murrumbidgee | M | 51,400 | 701,538 | 868,194 | 1,621,132 |
| EWK | Edward-Wakool (Edward and Wakool Rivers) | M | 94,800 | 386,885 | 288,076 | 769,761 |
| MID | Mid-Murray (Euston – Torrumbarry) | M | 5000 | 131,156 | 167,929 | 304,085 |
| LOD | Loddon-Pyramid | FD | 220,000 | 492,500 | 854,000 | 1,566,500 |
| GUN | Gunbower | M | 32,500 | 0 | 330,500 | 363,000 |
| CAM | Campaspe | FD | 60,000 | 277,000 | 717,000 | 1,054,000 |
| GOU | Goulburn | M | 0 | 60,870 | 922,150 | 983,020 |
| BRO | Broken | FD | 219,000 | 337,300 | 564,300 | 1,120,600 |
| BAR | Barmah (Tocumwal to Torrumbarry) | M | 0 | 162,049 | 110,382 | 272,431 |
| YAR | Murray (downstream Yarrawonga–Tocumwal) | M | 0 | 56,563 | 40,604 | 97,167 |
| OVE | Ovens | FD | 120,300 | 0 | 131,293 | 251,593 |
| — | Total | | 1,368,047 | 3,829,940 | 6,181,042 | 11,379,029 |

*Note:* Victorian stocking data were sourced from the Victorian Fisheries Authority website: https://vfa.vic.Gov.au/recreational-fishing/fish-stocking. Stocking records from New South Wales were provided by the New South Wales Department of Primary Industries.

broodstock with frequent replacement and a minimum number of broodstock required per consignment of fish (Rowland and Tully 2004; Rowland 2013; Hunt and Jones 2018). The government programme FishGen has also been developed recently to catalogue genomic data for broodstock to minimise inbreeding and maintain genetic diversity in hatcheries. Despite widespread stocking, golden perch stocks are still classified as 'Depleted' under the 'Status of Australian Fish Stocks Reports' in New South Wales and 'Depleting' in South Australia (Earl et al. 2023). Ongoing management of stocking for golden perch would benefit from knowledge of any historical or contemporary genetic implications of stocking for wild populations to inform strategies that enhance genetic resilience and long-term sustainability of the species.

Here, we used a combination of otolith-based age and chemistry data and genome-wide single-nucleotide polymorphism (SNP) data to explore the potential genetic effects of stocking on receiving populations of golden perch across the MDB. Because of the golden perch's high dispersal capacity and limited spatial population genetic structure (Booth et al. 2024), we used natal origin assignments based on otolith chemistry to identify stocked individuals (Zampatti et al. 2019, 2022). We integrated otolith and genome-wide SNP data to assess patterns of genetic substructure and kinship among stocked fish and evaluate the potential implications of stocking for genetic diversity and effective population size in receiving populations. We also used annual age estimates derived from otoliths to determine whether genetic signatures of stocking have changed through time. We outline how critical evaluation of stocking can inform fisheries management practices to promote the long-term sustainability of an important freshwater fish species in the wild in a way that balances conservation goals with recreational and economic needs.

## 2 | Materials and Methods

### 2.1 | Species Biology

Golden perch is a native freshwater fish species widespread in central-eastern Australia. Current taxonomy recognises only one species of golden perch. However, genetic divergence occurs across separate drainage basins (MDB, Fitzroy, Lake Eyre and Bulloo–Bancannia), which may represent distinct subspecies or species (Beheregaray et al. 2017; Attard et al. 2022). The present study is focused on the MDB golden perch. The golden perch is a medium-to-large (up to 760 mm), long-lived (up to 26 years) species that reaches sexual maturity at 2 and 4 years for males and females, respectively (Mallen-Cooper and Stuart 2003). Golden perch are highly fecund, with larger females (over 4 kg) producing up to 750,000 eggs per spawning event (a female of 2.2–2.4 kg typically produces around 500,000 eggs; Koehn and O'Connor 1990; Koehn et al. 2020). In rivers, spawning typically occurs in spring-summer but is linked to flow pulses and temperature cues (Balcombe et al. 2006; King et al. 2016; Koster et al. 2017). Spawning aggregations of adults will typically occur over short periods, though there may be several spawning events per season (Koehn et al. 2020). The species' life history operates over large spatial scales (hundreds to thousands of kilometres), often involving substantial flow-assisted passive dispersal of eggs and larvae and active dispersal of juveniles and adults (Reynolds 1983; Stuart and Sharpe 2020). Eggs can drift downstream for 1–2 days, and subsequent larval drift may occur for another 10–12 days, potentially over hundreds of kilometres (Koehn et al. 2020). Larvae may then settle in terminal wetlands or along channel edges.

### 2.2 | Hatchery Breeding Programmes and Stocking

Golden perch is highly fecund, and thus large numbers of offspring can be produced from a relatively few parental broodstock, highlighting the importance of genetic management of breeding programmes (Hunt and Jones 2018). To produce fingerlings, broodstock are collected from the wild and housed in semi-natural earthen ponds or tanks to acclimate. Spawning is induced via the use of injected hormones, and eggs are kept in incubation tanks until completion of hatching. Larvae are typically reared to fingerling stage (30–50 mm, 10–12 weeks) in earthen ponds prior to stocking. Under the New South Wales Hatchery Quality Assurance Program, larval rearing ponds are to be stocked with a batch of larvae that has a minimum of five contributing parental pairs. To maintain an equal contribution from each pair, the number of fish stocked into ponds is equalised for each pair. Broodstock are rotated to avoid same-pair matings, and they are replaced every 5 years to ensure genetic diversity of stocked fish (Rowland and Tully 2004; Rowland 2013; Hunt and Jones 2018).

### 2.3 | Sampling

Fin clip tissue samples were taken from a total of 559 golden perch from 22 river reaches across the MDB between 2015 and 2018 (Figure 1; Table 2). Samples were collected opportunistically during routine state and basin-wide fish monitoring programmes (e.g., see Crook et al. 2023). Fish were sampled using standardised single-pass boat electrofishing techniques. Most samples ($N = 448/559$) were collected between November 2017 and June 2018. Most individuals analysed were adults, with lengths ranging from 46 to 595 mm (median length 404 mm; Figure S1). A random subset of 366 of the golden perch sampled was euthanised and their otoliths removed to estimate age, natal origin and to reconstruct their movement history (see below). Individuals that were not euthanised for otoliths were released. Samples were collected in accordance with ethical guidelines and under the appropriate collection permits (Victoria: Victorian Fisheries Research Permit RP827, Fauna and Flora Guarantee Research Permit 10,007,273, Department of Environment, Land, Water and Planning Animal Ethics 14/04 and 19/008; NSW: NSW Animal Care and Ethics permit 14/10, Scientific Collection Permit P01/0059(A)-3.0 and Fisheries NSW Scientific Research Permit F93/158(C); Queensland: General Fisheries Permit 186,281 and Animal Care and Ethics CA 2016/01/938; and South Australia: under an exemption (No. 9902132) of Section 115 of the Fisheries Management Act 2007 and following the South Australian Animal Welfare Act 1985).

### 2.4 | Otolith Chemical Tracers to Identify Stocked Individuals

In an earlier study by Zampatti et al. (2019), otoliths for 279 of the 559 individuals sequenced in the present study were analysed for

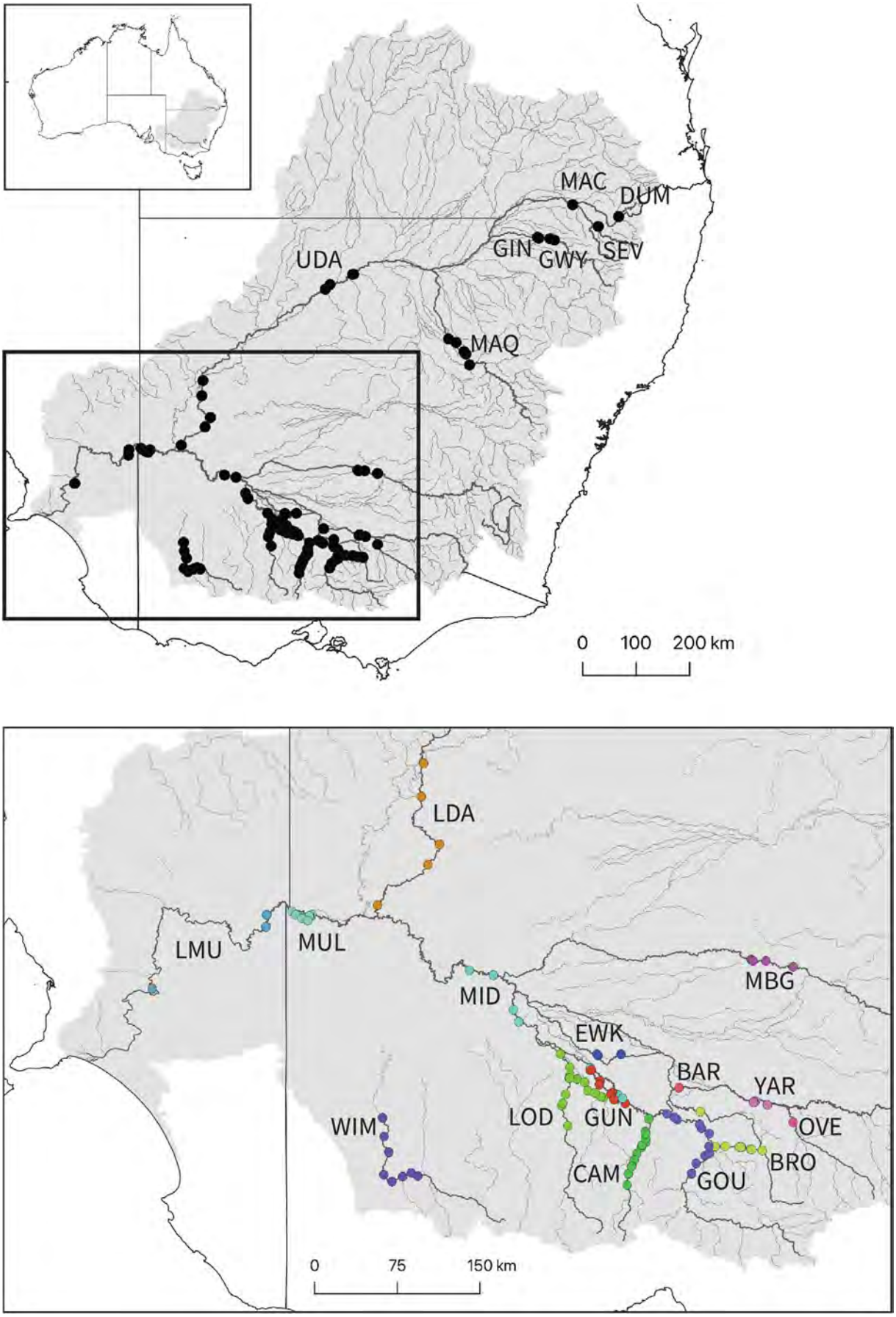


**FIGURE 1** | Legend on next page.

**FIGURE 1** | Map of the study region. Murray–Darling Basin (MDB) is shaded in grey. Major rivers and streams are in light grey, and major rivers sampled are in dark grey. Circles are individual sampling locations; circles in the lower panel are colour-coded by reach. River reach codes are in Table 1.

**TABLE 2** | Code for river reach (Code, matches that in Table 1); number of golden perch sequenced per river reach ($N_{sam}$); number of samples retained for genetic analysis post-filtering ($N_{gen}$); number of samples for which genetic and otolith age data were available ($N_{gen+age}$); number of samples for which genetic and otolith chemistry data were available ($N_{gen+chem}$); number and percentage (out of $N_{gen+chem}$) of individuals that were inferred as stocked based on otolith chemistry ($N_{stk\_chem}$); number and percentage (out of $N_{gen}$) of individuals that were inferred as stocked based on otolith chemistry and kinship ($N_{stk\_chem+gen}$); number of first-order kin pairs within the reach ($N_{FO}$); number of second-order kin pairs within the reach ($N_{SO}$).

| Code | $N_{sam}$ | $N_{gen}$ | $N_{gen+age}$ | $N_{gen+chem}$ | $N_{stk\_chem}$ | $N_{stk\_chem+gen}$ | $N_{FO}$ | $N_{SO}$ | $N_eLD$ | $N_{eColony}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| LMU | 20 | 20 | 14 | 8 | 1 (13%) | 1 (5%) | 0 | 0 | ∞ (∞–∞) | 2,147,483,647 (1–2,147,483,647) |
| MUL | 17 | 14 | — | — | — | 0 | 0 | 0 | — | — |
| LDA | 30 | 30 | 22 | 15 | 0 | 0 | 0 | 0 | ∞ (∞–∞) | 2,147,483,647 (1–2,147,483,647) |
| UDA | 30 | 30 | 29 | 26 | 1 (4%) | 1 (3%) | 0 | 2 | ∞ (739–∞) | 870 (340–2,147,483,647) |
| WIM | 23 | 23 | — | — | — | 4 (17%) | 12 | 2 | 48 (25–∞) | 40 (23–76) |
| MAQ | 40 | 40 | 40 | 39 | 29 (74%) | 33 (83%) | 11 | 12 | 89 (55–183) | 80 (52–130) |
| GIN | 26 | 25 | 22 | 15 | 2 (13%) | 6 (24%) | 7 | 1 | 101 (44–∞) | 100 (55–263) |
| GWY | 22 | 22 | 21 | 17 | 3 (18%) | 3 (14%) | 6 | 1 | 98 (37–∞) | 71 (41–157) |
| MAC | 20 | 20 | 20 | — | — | 1 (5%) | 1 | 4 | 202 (75–∞) | 253 (103–2,147,483,647) |
| SEV | 11 | 11 | 11 | — | — | 0 | 0 | 1 | — | — |
| DUM | 19 | 19 | 19 | — | — | 2 (11%) | 12 | 2 | 26 (12–110) | 26 (15–53) |
| MBG | 40 | 39 | 39 | 37 | 19 (51%) | 22 (56%) | 1 | 8 | 389 (169–∞) | 329 (191–979) |
| EWK | 11 | 11 | — | — | — | 1 (9%) | 0 | 1 | — | — |
| MID | 70 | 68 | 48 | 47 | 8 (17%) | 9 (13%) | 1 | 8 | 1193 (569–∞) | 759 (500–1738) |
| LOD | 27 | 27 | 14 | 15 | 9 (60%) | 11 (41%) | 1 | 3 | 284 (111–∞) | 281 (145–4694) |
| GUN | 20 | 20 | — | — | — | 2 (10%) | 0 | 2 | 2,825 (245–∞) | 760 (244–2,147,483,647) |
| CAM | 40 | 37 | 18 | 20 | 20 (100%) | 31 (84%) | 19 | 12 | 48 (31–86) | 53 (35–86) |
| GOU | 31 | 30 | 11 | 6 | 5 (83%) | 7 (23%) | 3 | 8 | 160 (75–∞) | 116 (76–236) |
| BRO | 25 | 25 | — | — | — | 6 (24%) | 9 | 8 | 37 (19–138) | 44 (26–81) |
| BAR | 9 | 6 | 6 | 6 | 2 (33%) | 2 (33%) | 0 | 0 | — | — |
| YAR | 27 | 27 | 20 | 20 | 11 (55%) | 11 (41%) | 0 | 3 | 439 (198–∞) | 468 (211–212,993) |
| OVE | 1 | 1 | 1 | — | — | 1 (100%) | — | — | — | — |
| Total | 559 | 545 | 355 | 271 | 110 | 154 | 83 | 78 | — | — |

*Note:* Effective population size estimates for the linkage disequilibrium (LD) method ($N_eLD$) and Sibship method ($N_{eColony}$). We applied the physical linkage and age structure bias corrections from Waples et al. (2014) to LD estimates to calculate the adjusted effective population size per generation. Effective population size estimates of 2,147,483,647 returned by colony reflected the lack of kin in the sample and should be considered equivalent to the ∞ value returned by the $N_eLD$ method.

strontium isotope ratios ($^{87}Sr/^{86}Sr$) using laser ablation inductively coupled mass spectrometry (LA-ICP-MS). Our classifications of individuals as wild or hatchery in origin were based on the natal origin assignments inferred in Zampatti et al. (2019). Natal origin assignments were derived from a Bayesian mixing isotope model (BMIM) and a Bayesian assignment approach outlined in Zampatti et al. (2019, 2022). In short, a $^{87}Sr/^{86}Sr$ isoscape map for the MDB main rivers was created from water samples (726 water samples collected across the MDB between 2011 and 2018) and then this map was used to probabilistically assign the stationary sections of $^{87}Sr/^{86}Sr$ profiles extracted from the otolith. If there was a change in otolith $^{87}Sr/^{86}Sr$ profiles during the 0–1-year period (when stocked fish are released into rivers), the assignment approach calculated the probability that the fish's natal $^{87}Sr/^{86}Sr$ signature had a hatchery $^{87}Sr/^{86}Sr$ signature compared to all possible rivers (penalised by distance). Except for MBG, hatchery probabilistic assignments were >99%. The MBG river is stocked with fish from hatcheries with partially overlapping $^{87}Sr/^{86}Sr$ signatures, and probabilistic assignments were less confident at >85%. Additional details on otolith chemical tracer methods are provided in Supporting Information S1: Data S1.

### 2.5 | Age and Growth Increments to Determine Whether Effects of Stocking Have Changed Through Time

Age estimates and annual growth increment measurements from otolith samples were obtained for 363 of the 559 individuals sequenced in this study using the service provider Fish Ageing Services (FAS). These 363 individuals included 276 of the 279 individuals whose otoliths were analysed for strontium isotope ratios. Additional details on ageing methods are provided in Supporting Information S2: Data S1.

### 2.6 | Chromosome-Length Genome Assembly

To support genotyping, we generated a chromosome-length genome assembly based on an existing draft genome assembly for the MDB golden perch lineage (GenBank assembly accession number: GCA_008360985.2; Pavlova et al. 2022). A Hi-C library was constructed using a frozen fin tissue sample, following the methods outlined in Rao et al. (2014). A total of 100,680,198 M Hi-C reads were generated using NovaSeq 6000 (Illumina). Library preparation and sequencing were performed by the DNA Zoo Consortium (https://www.dnazoo.org/). The draft genome was scaffolded to 24 chromosomes (3345 scaffolds, scaffold N50 of 28.1 Mb, longest scaffold 35.0 Mb) using 3D-DNA (Dudchenko et al. 2017) and Juicebox Assembly Tools (Dudchenko et al. 2018), according to methods described at https://www.dnazoo.org/methods. The chromosome-level genome assembly (GOP001_1.1_HiC) is available on the DNA Zoo website along with interactive Hi-C contact maps before and after the Hi-C-guided assembly (https://www.dnazoo.org/assemblies/Macquaria_ambigua).

### 2.7 | Sequencing and Genotyping

A total of 559 individuals were sequenced using DArTseq, a method similar to double-digest restriction-associated sequencing (ddRAD). We genotyped individuals by mapping raw Illumina read data returned from DArT to the chromosome-level genome assembly. Details of the sequencing, genotyping pipeline and filtering steps are provided in Supporting Information S3: Data S1. The final dataset post-filtering consisted of 545 individuals genotyped at 2905 biallelic SNPs. Of the genotyped individuals retained for analysis, 355 (65%) also had otolith age data and 271 (50%) had otolith chemistry data. A total of 268 (49%) genotyped individuals had associated otolith age and chemistry data.

### 2.8 | Kinship Analysis

First-order and second-order kin pairs were identified using kinship analysis implemented in *Colony2* v2.0.6.8 (Jones and Wang 2010). Details of the analysis and run settings are provided in Supporting Information S4: Data S1. Our analysis included multiple overlapping generations, and not all individuals were aged. Thus, first-order kin pairs involving unknown age individuals could be either full-sibling or parent–offspring pairs. Second-order relationships may represent half-sibling, aunt/uncle–niece/nephew, or grandparent–grandchild relationships.

### 2.9 | Integrating Otolith Chemistry and Genetic Kinship Data to Infer Natal Origin

We used the kinship data to fill gaps in the otolith chemistry data and assign natal origin to additional individuals. Among the 139 individuals assigned to first-order kin groups, there were 40 individuals that did not have natal origin assignments but that had first-order kin for which natal origin assignments were available. Given that in 77% (20/26) of cases where there was more than one individual per kin group, the individuals were the same age or within 1 year of age; first-order kin groups were assumed to reflect likely true full-sibling relationships. Thus, first-order kin assignments were used to assign the same natal origin (i.e., stocked or wild) to all individuals in a first-order kin group. Although it is possible for the natal origin of first-order kin to differ (e.g., because breeding adults are taken and returned to the wild), sampling this type of parent–offspring pair was unlikely due to the small number of hatchery broodstock relative to the large wild population size. Natal origin in all subsequent analyses was based on the integrated otolith chemistry and kinship assignments. Additional details and justification of the integration of otolith and kinship data are provided in Supporting Information S5: Data S1.

### 2.10 | River Distances Between Kin

We calculated river distances between the capture locations of first-order kin pairs and second-order kin pairs along the Geofabric Surface Network (http://www.bom.gov.au/water/geofabric/) using the *sfnetworks* package (van der Meer et al. 2023) in *R* v 3.5.1 (R Core Team 2018) to determine the spatial scale of movement for stocked and wild-origin kin. Because the Wimmera (WIM) river system is not connected hydrologically to the rest of the rivers sampled, even during flood events, river distances were not calculated for pairs sampled across WIM and a different river.

## 2.11 | Genetic Diversity

Population-level estimates of mean observed heterozygosity (Ho) and gene diversity (Hs) were calculated for each river reach using the *Hierfstat* package (Goudet 2005) in *R*. Population-level genetic diversity statistics were not estimated for the Ovens (OVE) River due to small sample size ($N < 5$). Individual heterozygosity (PHt; proportion of heterozygous loci per individual), which is expected to be inversely correlated with the degree of individual inbreeding (Kardos et al. 2015), was calculated using the *Genhet* package (Coulon 2010) in *R* (Kardos et al. 2015).

## 2.12 | Effective Population Size

Effective population size ($N_e$) was estimated for each river reach when the number of sampled fish was greater than 15 using the sibship method implemented in *Colony2* (with the same settings as described above) and the linkage disequilibrium (LD) method in *NeEstimator* (Do et al. 2014). The LD method was implemented assuming random mating, removing singleton alleles and calculating 95% confidence intervals using the jackknife-across-samples method. For a single cohort of an iteroparous species, the LD method estimate reflects the effective number of breeders in one breeding cycle ($N_b$; considered to reflect short-term $N_e$ and inbreeding in the parental generation). For a mixed age sample, the LD method estimates will reflect the effective size per generation ($N_e$; important for long-term evolutionary processes) and will typically be downward biased due to mixture LD from age structure (Waples et al. 2014). LD method estimates for each river were adjusted to correct for the downward physical linkage bias (using the haploid number of 24 chromosomes (Shams et al. 2019)) and age structure bias using two life-history traits (adult lifespan (AL) and age at maturity ($\alpha$)), following the methods outlined in Waples et al. (2014). We set $\alpha$ to 3, given the age at maturity is 2 and 4 years for males and females, respectively (Mallen-Cooper and Stuart 2003). Given a longevity of ~26 years, we set AL to 23 ($26 - \alpha$). There is currently no correction available for *Colony2* Ne estimates.

Single cohort estimates of $N_e$ are expected to give more precise estimates than mixed age samples comprising many cohorts (Bertram et al. 2024). For fish of known age that were assigned a natal origin based on integrated otolith chemistry and genetic kinship data ($N = 277$), we calculated $N_e$ for different binned cohorts of stocked versus wild fish to compare wild and stocked population samples and look for evidence of temporal changes in $N_e$ through time. Binned cohorts reflected fish born pre-2009, 2009–2010, 2011–2012 and 2013–2016; the size of bins was chosen to avoid sample sizes $< 20$ as small sample sizes can affect the robustness of $N_e$ estimates while still allowing an estimation of change through time.

## 2.13 | Population Genetic Substructure

We tested for signatures of population substructure in the whole genetic dataset that may be associated with stocking. Population genetic structure patterns were assessed using a Pearson principal component analysis implemented using the gl.pcoa function in the dartR package in R and *Structure* (Pritchard et al. 2000; Falush et al. 2003). *Structure* was run for *K* values 1–12 (10 replicate runs per *K*) using the admixture model with correlated allele frequencies and uninformative location priors. Runs were 100,000 Markov Chain Monte Carlo iterations, after an initial burn-in period of 50,000 repetitions. We used the main CLUMPAK pipeline to summarise *Structure* runs and the 'Best K' pipeline to implement the Evanno et al. (2005) method to assess the number of genetic clusters that best represented the different hierarchical levels of population substructure present in the data (Kopelman et al. 2015). *Structure* results were visualised using *Structure Plot* (Ramasamy et al. 2014). We tested whether there was an association between the degree of genetic substructure and natal origin using a hierarchical Bayesian regression model fitted in *R* using the stan_glmer function in the rstanarm *R* package (Goodrich et al. 2020; details in Supporting Information S6: Data S1). We also tested for an association between the number of fish stocked (1990–2019) in a river and degree of genetic substructure using the stan_lm function using the rstanarm *R* package (details in Supporting Information S6: Data S1).

## 2.14 | Temporal Genetic Substructure Patterns

We tested for changes in patterns of genetic substructure (a proxy for the presence of stocked fish, statistically supported by an association between natal origin and individual admixture coefficients and by an association between stocking intensity and individual admixture coefficients in *Structure* analysis) over time using a hierarchical Bayesian regression model fitted in R using the *stan_glmer* function in the *rstanarm* R package (Goodrich et al. 2020). We fitted a model where the response was the individual admixture coefficient for the primary genetic cluster (Q1) from the *Structure* analysis and the predictor was birth year based on otolith age (standardised to a mean of zero and SD = 1). River reach and sibling group were included as random effects to account for population effects and the inclusion of close kin. The model was fitted with a Beta likelihood with a logit link function (given the response variable ranged from 0 to 1). Default priors were used, and the target proposal acceptance rate (adapt_delta) was set to 0.95. Posterior distributions were based on four chains of 10,000 Markov Chain Monte Carlo iterations. Model convergence and mixing were assessed using the Gelman–Rubin statistics and the effective number of samples. All Gelman–Rubin statistics were near one, and effective samples were > 1000 for all examined parameters. We report 95% and 80% credible intervals to ascribe support to observed effects.

## 2.15 | Testing for Evidence of Cross-Basin Hybridisation

Our study focused on golden perch in the MDB. However, cross-basin hybridisation in the MDB has been reported in previous genetic studies (Beheregaray et al. 2017; Attard et al. 2022). To test whether individuals associated with very high individual heterozygosity values (see Genetic Diversity) were hybrids, we looked for evidence of cross-basin hybridisation with

divergent golden perch that occur in separate drainage basins (Fitzroy, Lake Eyre and Bulloo–Bancannia drainage basins; Beheregaray et al. 2017; Attard et al. 2022). This was done based on a separate co-analysis of our DArTseq data with pre-existing DArTseq data for samples representative of the Fitzroy, Lake Eyre Basin and Bulloo–Bancannia drainages (see Supporting Information S7: Data S1). Briefly, we used principal component analysis to visualise broad spatial structure among basins. We used ADMIXTURE (Alexander et al. 2009) with both unsupervised and supervised learning modes to estimate cross-basin ancestry coefficients and plotted the results using the *pophelper* R package (Francis 2017). We then assessed the power of the SNP data to distinguish among simulated hybrid classes and tested the empirical data using the *hybriddetective* R package (Wringe et al. 2017), defining pure reference samples as those with > 99% ancestry to a single basin and potential hybrids as those with at least 10% MDB and 10% ancestry from another basin based on the unsupervised $K = 5$ admixture results. Finally, we estimated the hybrid index (Buerkle 2005) for non-reference samples from the MDB with the *gghybrid* R package (Bailey 2024), and generated triangle plots to visualise the relationship between interspecific heterozygosity and hybrid index using the *introgress* R package (Gompert and Buerkle 2010; see Supporting Information S7: Data S1).

## 3 | Results

### 3.1 | Kinship Analysis

Kinship analyses revealed many close kin, with first-order kin pairs detected in 55% (12/22) of the rivers sampled (Table 2). A total of 139/545 (26%) individuals genotyped were assigned to at least one first-order relative (full-sibling or parent–offspring relationship; Table S2). These first-order relatives comprised 49 first-order kin groups ranging from 2 to 5 individuals (Table S3). Some rivers had comparatively high proportions of first-order kin detected; 39%–53% of individuals genotyped in BRO, CAM, MAQ, DUM and WIM were assigned a first-order relative in the same reach (Table 2). First-order kin groups were not always sampled together in the same location at the same time; of the first-order kin groups identified, 57% (28/49) were in multiple (up to three) river reaches (Table S3) and 43% (21/49) were represented across multiple (up to two) field seasons (Table S4). Of the 29 first-order kin groups where otolith age data were available for two or more individuals, 66% (19/29) contained full-sibling relationships where at least two individuals in the group were the same age (Table S5). Ten of the first-order kin groups contained individuals of varying ages (three of which also included multiple individuals of the same age; Tables S5 and S6); In 4 (out of 10) of the mixed-age first-order kin groups, individuals differed in age by only 1 year, indicative of either possible repeat breeding events by the same parents in consecutive years (in either the wild or hatchery environment) or small ageing errors, suggesting that fish are actually siblings (Table S6). In the remaining 6 (out of 10) mixed-age first-order kin groups, larger age discrepancies could also represent possible parent–offspring relationships (Table S6). For 31% (15/49) of kin groups, age data were available for only one individual, and for the remaining 16% (8/49), age data were not available for any individuals (Table S5).

Second-order kin pairs were pervasive, with 50% (272/545) of individuals genotyped assigned to at least one second-order relative (Tables 2 and S7). A total of 410 second-order kin pairs (half-sibling, aunt/uncle–niece/nephew or grandparent–grandchild relationships) were identified. The majority, 81% (332/410) of these second-order kin pairs, were between-reach pairs, sampled across two different river reaches (Table S7).

### 3.2 | Integrating Otolith Age and Chemistry and Genetic Kinship Data to Infer Natal Origin

A total of 41% (110 out of 271) of the individuals with otolith chemistry data were previously classified as stocked in origin based on $^{87}Sr/^{86}Sr$ (Table S8). Integrating the otolith chemistry and kinship data identified an additional 40 individuals that were likely of hatchery origin (Table S8; Supporting Information S5: Data S1). Stocked individuals were detected in all rivers, except MUL, LDA and SEV (Table 1). More than 50% of individuals genotyped in MAQ, MBG and CAM were stocked (Table 2). The age range of stocked individuals was 1–20 years (mean = 6.78, SD = 4.07). The age range of inferred wild individuals was 1–24 years (mean = 7.43, SD = 4.06).

### 3.3 | Prevalence of Kin in Stocked Individuals

Stocked individuals were associated with a higher prevalence of close kin compared with wild-origin individuals: the majority (63%; 31/49) of the first-order kin groups were identified as stocked in origin (i.e., had at least one individual assigned as stocked in origin; Tables S2 and S8). Of the remaining 18 first-order kin groups, only 6 (12%) were inferred to be wild in origin and the remaining 12 (25%) were of unknown origin (Tables S2 and S8). Stocked first-order kin groups were comprised of individuals located in both the same (39%; 12/31) and across multiple (61%; 19/31) river reaches. Three of the six wild-origin, first-order kin groups consisted of individuals sampled across multiple rivers separated by up to 680 km of river channel (Tables S2 and S8).

Of the 410 second-order kin pairs identified, 79% (323 pairs) included at least one stocked individual. This included 227 pairs with only one individual assigned as stocked, 96 pairs with both individuals assigned as stocked and 30 pairs with one individual assigned as stocked and one individual assigned as wild (Table S7). For the pairs where both individuals were identified as stocked, 21% (20/96) were in the same river reach and 79% (76/96) were in two different reaches. There were only 10 second-order pairs where both individuals were identified as wild origin (five were in the same reach; five were in different reaches). There were 45 second-order kin pairs where natal origin was unknown for both individuals.

### 3.4 | River Distances Between Kin

Many (43%; 62/145) first-order kin pairs were detected across two river reaches (Figure 2; Tables S2 and S10). The highest numbers of between-reach first-order kin pairs were detected between the geographically proximate GOU and BRO ($N = 11$) and CAM and

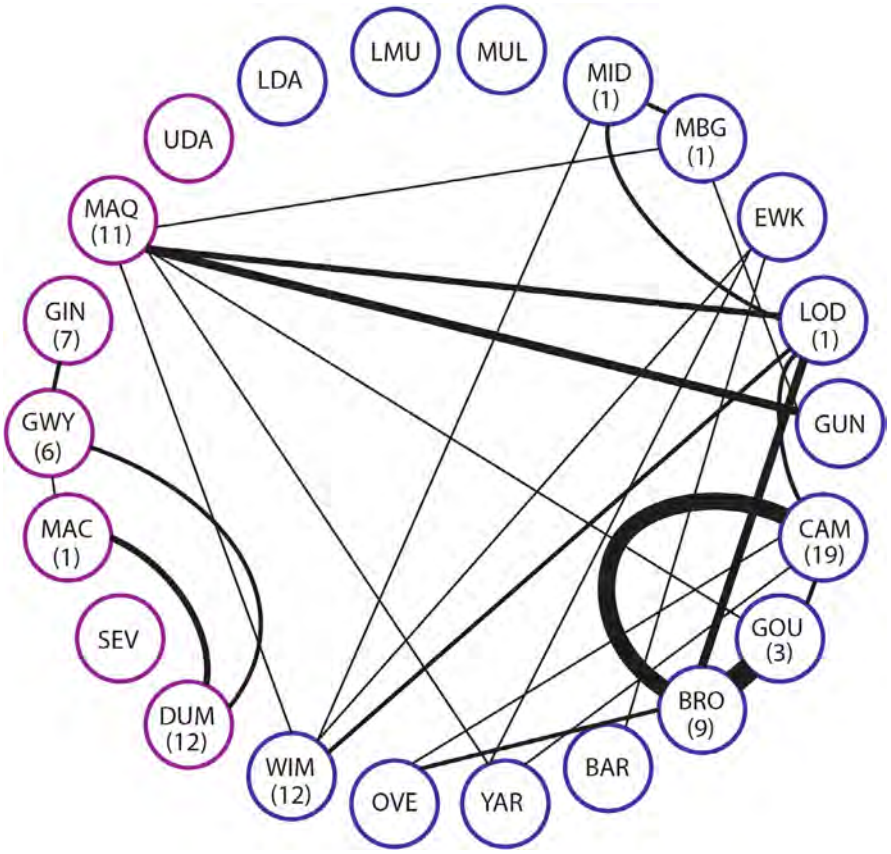


**FIGURE 2** | Network diagram representing first-order kin pairs (full-sibling or parent–offspring relationships) of golden perch detected between river reaches. The thickness of line is indicative of the number of sibling pairs. River reaches are arranged geographically; reaches in the northern parts of the Murray–Darling Basin are in purple, and reaches in the southern parts of the MDB are in blue. Numbers of within-reach first-order kin pairs are given in parentheses.

BRO ($N=8$) river systems (Figure 2; Table S10). River distances between first-order kin pairs ranged from 0 to 2665 km (median = 52 km; Figure S3a). All 10 first-order kin pairs separated by > 700 km were inferred to be stocked in origin. Only 5/13 of the wild first-order kin pairs were caught at different sites (including different sampling sites within the same river reach), with river distances for these pairs ranging from 4.3 to 680 km (median = 87.3 km). These wild origin pairs were between GUN and MBG (680 km; one pair); EWK and YAR (279 km; one pair); MID and MID (68 km; one pair); and GIN and GWY (4.3 km; two pairs).

Of the 332 between-reach second-order kin pairs, the highest numbers were detected between the MAQ and BRO ($N=17$), GOU and BRO ($N=14$), BRO and YAR ($N=13$) and WIM and MAQ ($N=13$) rivers (Table S11). River distances between second-order kin pairs ranged from 0 to 3018 km (median = 374 km; Figures S3b,c). There were 92 second-order kin pairs separated by very large distances (> 2000 km), the majority (84/92) of which had either both individuals (38/92) or one individual (46/92) inferred as stocked in origin. None of the 92 second-order pairs separated by > 2000 km had both individuals in the pair inferred as wild in origin. There were only 7 second-order kin pairs detected in different sites (including different sites within the same river reach) where both individuals were inferred to be wild in origin and river distances for these pairs ranged from 4.3 to 512 km (median = 213 km).

### 3.5 | Genetic Diversity

Differences in heterozygosity among populations were small and mostly non-significant (Figure S4). The mean observed heterozygosity (Ho) and gene diversity (Hs) values per river reach ranged from 0.22 to 0.27 and 0.22 to 0.23, respectively (Table S12). The mean individual heterozygosity values (PHt) per reach ranged from 0.22 to 0.27 (Table S12). GIN and GWY were associated with the highest genetic diversity across all metrics (Table S12). However, high genetic diversity estimates for GIN and GWY were highly sensitive to the inclusion of a group of individuals associated with high individual heterozygosity values (outlier PHt values ranged from 0.32 to 0.44; Table S15; details of high heterozygosity individuals are in Supporting Information S8: Data S1). High heterozygosity individuals included males and females, stocked and wild origin individuals, and did not appear to be cross-drainage hybrids (Supporting Informations S7 and S8: Data S1).

### 3.6 | Effective Population Size

Estimates of effective population size ($N_e$) for river reaches ranged from 26 to ∞ for both the LD and colony methods (Table 2; Figure 3). The infinite $N_e$ estimates obtained for LMU and LDA (consistent across both methods) and for UDA (LD method only) were indicative of $N_e$ indistinguishable

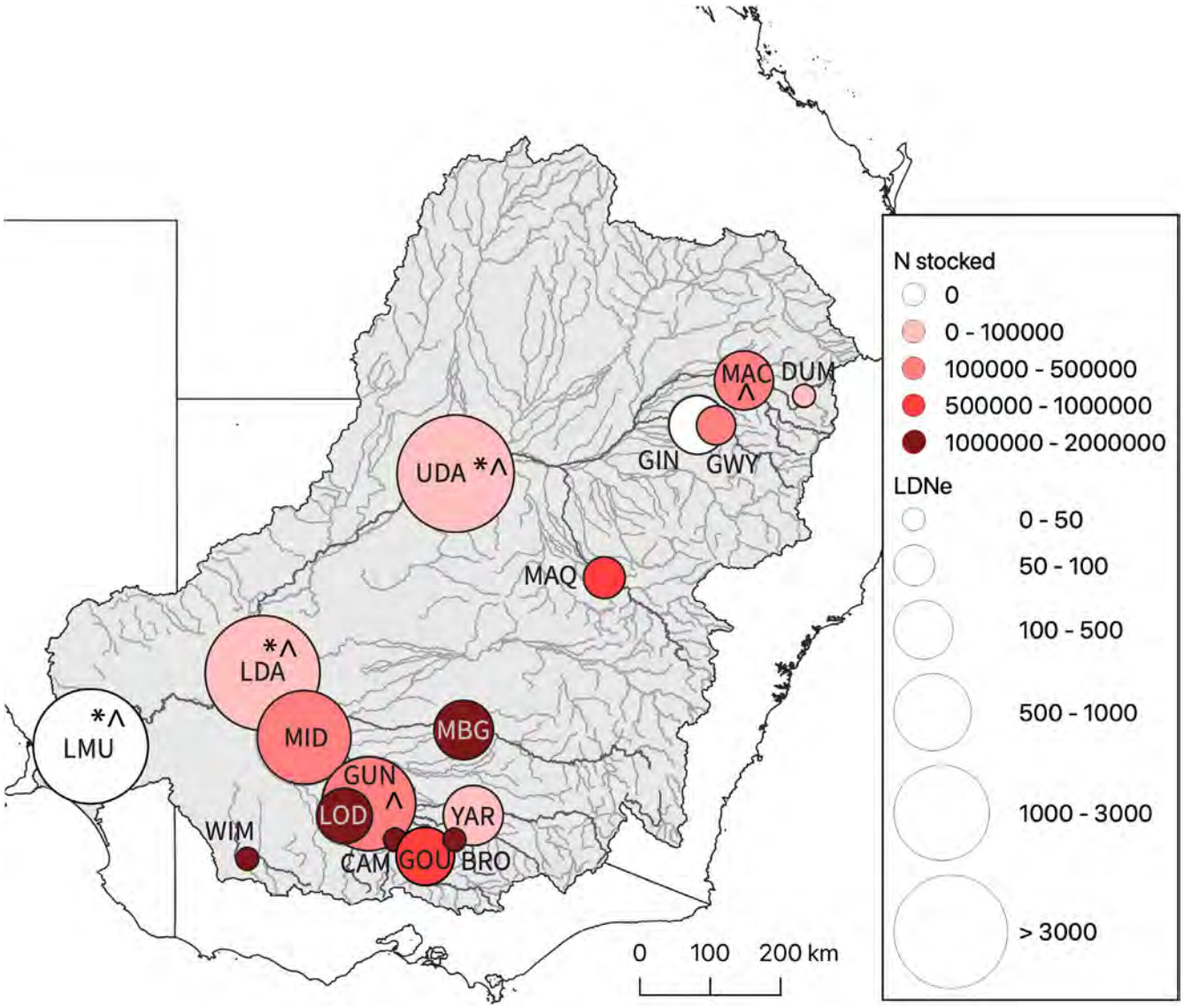


**FIGURE 3** | Colour scale represents the number of golden perch stocked directly into the sampled reach from 1990 to 2019 (i.e., intensity of stocking). Size of the circle represents the effective population size ($N_e$). Plotted Ne values are the corrected LD method estimates. *Indicates that $N_e$ estimates returned by the LD method were infinite. ^Indicates that the upper credible intervals for both the LD and colony methods spanned infinity.

from infinity (Gilbert and Whitlock 2015; Waples et al. 2016; Nadachowska-Brzyska et al. 2021; Table 2). Many of the LD method $N_e$ estimates had upper confidence intervals spanning to infinity (Table 2). However, we were able to obtain finite colony $N_e$ estimates (with finite credible intervals) for 12/17 of the locations, and these were highly correlated with LD $N_e$ estimates ($R^2 = 0.94$, $p < 0.05$; Table 2). Upper credible intervals spanning to infinity were obtained across both methods for five locations (LMU, LDA, UDA, MAC and GUN), indicative of very large $N_e$ (Table 2). Estimates of $N_e$ were < 100 (consistent across both methods) for several reaches (WIM, MAQ, GWY, DUM, CAM, BRO; Table 2). As for genetic diversity estimates above, the $N_e$ estimates for GIN and GWY were sensitive to the inclusion of individuals with high individual heterozygosity values (Table S15; details of high heterozygosity individuals are in Supporting Information S8: Data S1).

The $N_e$ estimates for all binned cohorts of individuals inferred to be stocked were lower than those for inferred for wild origin individuals (stocked $N_e$ range = 63–156; wild $N_e$ range = 494–∞; Table 3). For inferred stocked individuals, $N_e$ estimates were highest for the Pre-2009 binned cohort (although this bin consisted of the largest number of cohorts: individuals born 1993–2008). For inferred wild origin binned cohorts, $N_e$ estimates of infinity for the 2013–2016 and pre-2009 bins (consistent across both methods) were likely indicative of $N_e$ estimates too large to estimate rigorously with the available sample size (Table 3).

### 3.7 | Genetic Substructure Patterns

Much of the genetic substructure evident in both PCA and structure analysis was driven by the large number of close kin in the dataset, with genetic clusters in PCA and *Structure* aligning closely with first-order kin groups (Figures S7–S11). Overall, there was little evidence of spatial genetic structure across sampled rivers in the MDB (Figure S12). Based on *Structure* analysis ($K = 7$), most individuals in the downstream reaches of the

**TABLE 3** | Effective population size estimates per binned cohort for stocked and wild origin golden perch pooled across all sampled river reaches.

| Cohorts | *N* | $N_e$LD | $N_{eColony}$ |
|---|---|---|---|
| Stocked | | | |
| Pre-2009 (1997–2008) | 34 | 156 (74–5583) | 150 (92–301) |
| 2009–2010 | 21 | 91 (48–406) | 93 (52–224) |
| 2011–2012 | 28 | 63 (33–287) | 72 (45–133) |
| 2013–2016 | 40 | 87 (50–223) | 87 (57–139) |
| Wild | | | |
| Pre-2009 (1993–2008) | 32 | ∞ (712–∞) | 2,147,483,647 (1–2,147,483,647) |
| 2009–2010 | 52 | ∞ (1809–∞) | 1768 (850–205,121) |
| 2011–2012 | 39 | 821 (229–∞) | 494 (260–3323) |
| 2013–2016 | 31 | ∞ (∞–∞) | 2,147,483,647 (1–2,147,483,647) |

*Note:* Estimates are from the linkage disequilibrium method ($N_e$LD) and the Sibship method ($N_{eColony}$). We applied the physical linkage bias correction from Waples et al. (2014) to all $N_e$LD estimates. We applied the age structure bias correction from Waples et al. (2014) to $N_e$LD estimates for the Pre-2009 cohort (because it represented a large range of ages). Ne estimates of 2,147,483,647 returned by colony reflected the lack of kin in the sample and should be considered equivalent to the ∞ value returned by the $N_e$LD method.

Murray and Darling river systems (LMU, MUL, LDA) were strongly assigned to the primary cluster (mean individual assignment to Q1 > 0.95; Figure S12). Other river reaches throughout the MDB (e.g., WIM, MAQ, DUM, LOD, CAM, GOU, BRO) had strong signals of individuals being assigned partially or fully to clusters other than the primary cluster (Q1) (Figure S12).

Based on *Structure* analysis ($K = 7$), most wild origin individuals were assigned to the primary genetic cluster (Q1; orange cluster; Figure 4), with little evidence of assignment to other genetic clusters, whereas full and partial assignment to other clusters was more common in stocked fish and those of unknown origin (Figure 4). Bayesian regression analysis found a strong association between assignment to the primary genetic cluster (Q1) and natal origin (Figure S6). Based on this association, we treated high proportional assignment to Q1 as a proxy for wild natal origin and high proportional assignment to clusters other than Q1 as a proxy for hatchery natal origin. For example, an individual assigned to Q1 with an individual admixture proportion value less than 0.75 had a 98% chance of having a hatchery natal origin (details in Supporting Information S6: Data S1). Assignment to Q1 was also negatively correlated with the intensity of stocking (number of fish stocked; mean scaled estimate equal to −0.09 [90% CrI −0.13, −0.04]).

### 3.8 | Temporal Genetic Substructure Patterns

We found strong evidence (95% Bayesian credible support) that the assignment of individuals to the primary genetic cluster (Q1; proxy for wild natal origin, as supported by Bayesian regression analysis above) has decreased with time (Figures 5 and S13). Our finding of increasing signatures of the kin-associated genetic substructure (assignment to clusters other than Q1) over time, combined with positive associations between genetic substructure and hatchery natal origin and stocking intensity, suggests that the numbers of stocked kin in the MDB have increased, particularly from 2006 onwards (Figure 5).

## 4 | Discussion

Our study demonstrates the value of a general approach combining genetic and otolith (age and chemistry) datasets to explore the genetic implications of stocking in a widespread non-salmonid fish characterised by high genetic connectivity. Kin were detected in most rivers across the expansive MDB, and by integrating otolith-derived natal origin data, we revealed that most close kin were stocked in origin. Rivers with many stocked close kin were associated with very low effective population size estimates ($N_e < 100$). Furthermore, signatures of kin-associated population genetic substructure across the MDB (assignment to genetic clusters other than Q1 in Structure analysis) were associated with stocking and have increased over the last three decades (1991–2016), concomitant with an increase in the release of hatchery-produced fish (Hunt and Jones 2018) and reduced frequency of natural recruitment events during the Millennium drought (2001–2009; Zampatti and Leigh 2013). Although genetic evidence of stocking (kin-associated genetic substructure) was most pronounced in rivers that are, for management purposes, considered largely functionally disconnected by barriers (dams/weirs) from the larger wild metapopulation (e.g., reaches of the CAM, WIM, BRO, LOD, MAQ, DUM), it was not limited to these systems (Table 1; Figure S12). Our findings suggest there is scope to modify current stocking practices to enhance the effective population size of fish being stocked into interconnected riverine systems and promote the long-term persistence of golden perch in the wild.

### 4.1 | Genetic Signatures of Stocking Against a Background of High Natural Genetic Connectivity

The lack of spatial genetic structure detected in our study corroborates previous reports of high genetic connectivity across most of the MDB (Faulks et al. 2010; Attard et al. 2018, 2022; Booth et al. 2024). Some spatial genetic structure has previously been reported for the Paroo River and Lower Lakes region in the MDB; however, these areas were not sampled in this study (Beheregaray et al. 2017; Attard et al. 2018; Booth et al. 2024). High genetic connectivity is also consistent with expectations based on golden perch biology (e.g., capacity for long-distance passive and active movements over hundreds of kilometres at multiple life stages; Zampatti et al. 2018; Koehn et al. 2020; Stuart and Sharpe 2020, Thiem et al. 2022). We were able to detect wild origin first-order and second-order relatives separated by hundreds of kilometres (up to 680 km in river distance). Examples of kin pairs being detected over such larger riverine distances are rare in the literature for fish, but full siblings and half-siblings have been detected up to 85 km apart and 290 km apart, respectively, for Murray cod *Maccullochella peelii* larvae in the MBD (Furlan et al. 2024).

Despite high background genetic connectivity, we detected strong signatures of population genetic substructure associated with

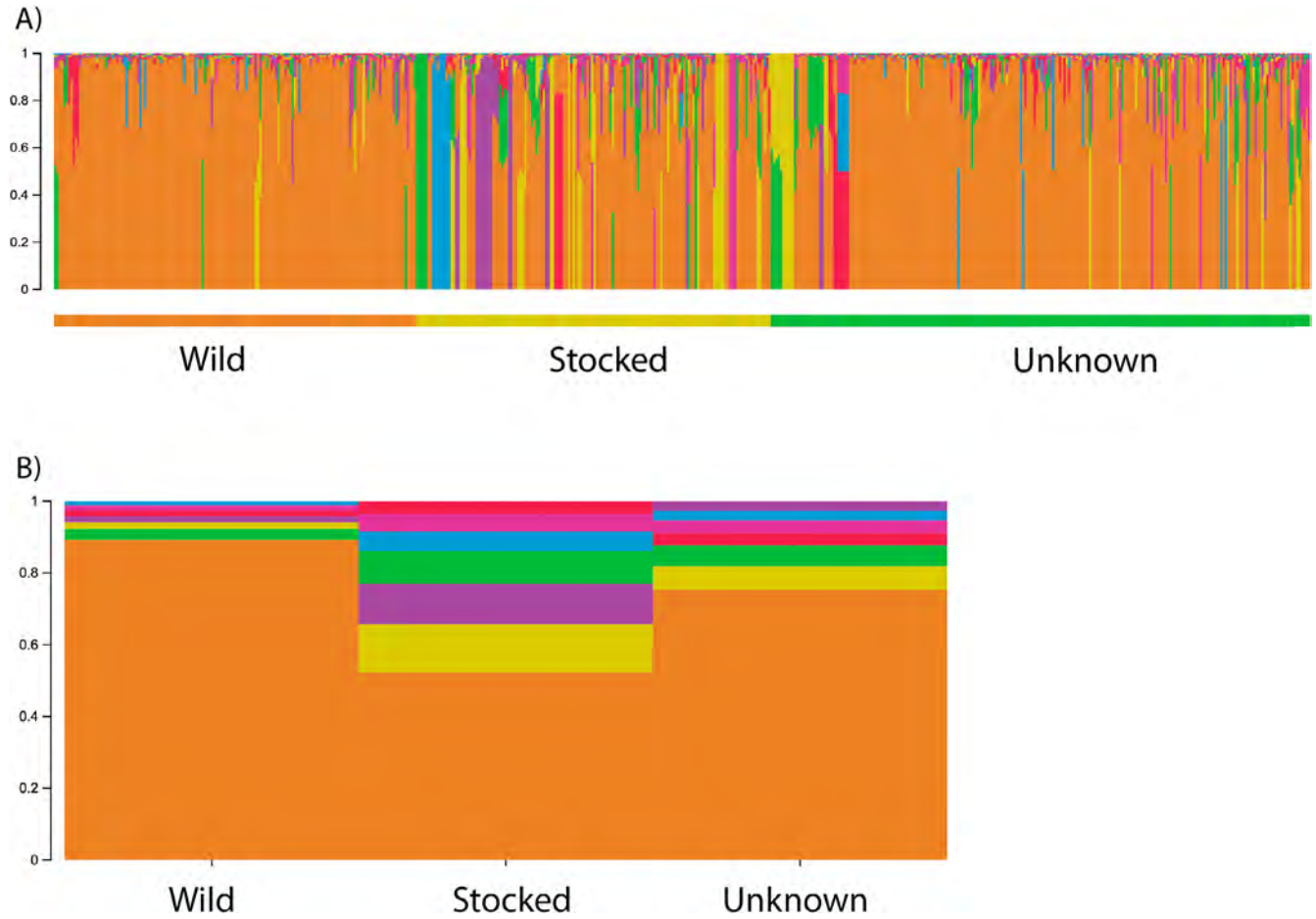


**FIGURE 4** | Summary of results of STRUCTURE analysis for $K = 7$ in golden perch from the Murray–Darling Basin. In panel (A), individuals are plotted as columns and are arranged along the X axis according to their natal origin as 'wild', 'stocked' or 'unknown' (inferred based on integrated otolith and genetic data). The *Q* values on the Y-axis represent the proportional individual assignment (A) and mean individual assignment per natal origin category (B) to each of the seven genetic clusters in the Structure analysis. Assignment to the primary genetic cluster (Q1; orange genetic cluster) was associated with an individual being of 'wild' origin.

the presence of many close kin among stocked individuals (both within and across river systems). Correlations between stocking intensity and signatures of admixture and modified genetic structure have been reported elsewhere for different species, mostly for salmonids, and mainly where non-local broodstock or domesticated broodstock were used (Hansen et al. 2009; Marie et al. 2010; Le Cam et al. 2015; Rougemont et al. 2019). Our study provides an example of genetic signatures of stocking being detectable, even when stocking programmes use wild-caught broodstock from a largely connected metapopulation.

### 4.2 | Genetic Evidence of Stocking Varies According to Local Context

Genetic evidence of stocking was detected in all rivers sampled but was more or less pronounced according to local context. Low effective population size ($Ne < 100$) and signatures of kin-associated population genetic substructure were most prevalent in river reaches considered, for management purposes, to be largely functionally isolated due to the presence of artificial barriers to movement (dams and weirs) that restrict immigration (e.g., reaches of the CAM, WIM, BRO, LOD, MAQ, DUM, GWY; Zampatti et al. 2019; Butler et al. 2024). These reaches are also considered by management to have limited natural spawning and recruitment and thus are reliant on stocking for local persistence. Consistent with our findings, previous studies have identified a high retention of stocked golden perch in barrier-laden systems and limited retention of stocked individuals in connected systems that facilitate immigration or natural spawning and recruitment (Hunt et al. 2010; Crook et al. 2016; Forbes et al. 2016; Thiem et al. 2017). However, genetic signatures of stocking (kin-associated population genetic substructure) were still evident to a lesser extent in river reaches considered part of the connected metapopulation and associated with natural recruitment, for example, the Murray River (MID, YAR) and its major tributaries (GOU, MBG). Genetic signatures of stocking were least evident in the Darling River (LDA, UDA) and the lower region of the Murray River (LMU), where the mean individual assignment to the primary genetic cluster (Q1 in Structure analysis; proxy for wild natal origin) was $\geq 0.95$. No first-order kin pairs were identified within these river reaches, and very few stocked fish were detected, consistent with the absence of direct stocking in the lower parts of the Murray system (LMU) and relatively low levels of stocking in the LDA and UDA (Table 1; Figure 3). Effective population size estimates were indistinguishable from infinity in these reaches,

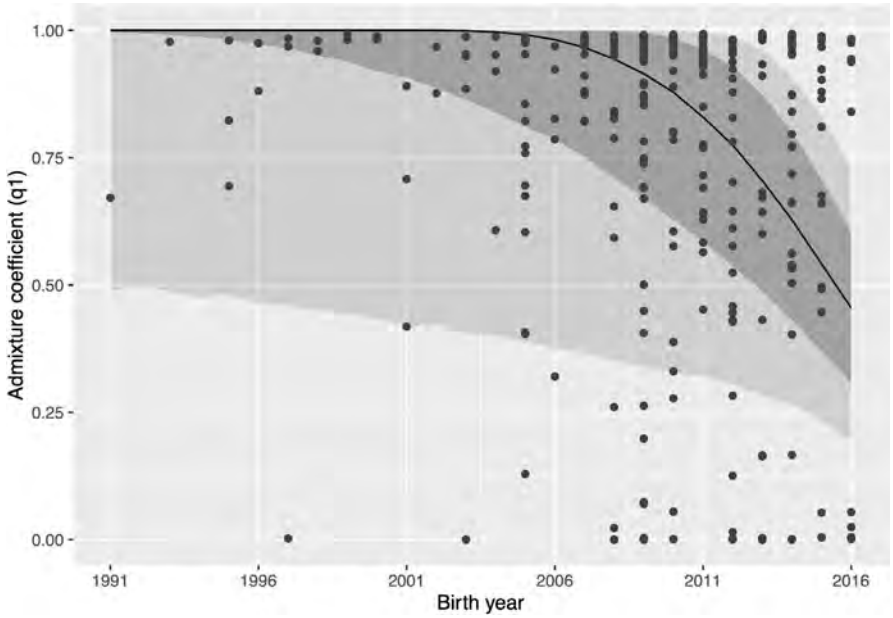


**FIGURE 5** | Results of hierarchical Bayesian regression model to explore changes in patterns of kin-associated population genetic substructure (a proxy for stocking effects) in golden perch over time. Plot shows fitted relationship between birth year of individuals and their admixture coefficient for the primary genetic cluster (q1; proxy for wild origin) in the Structure analysis. Light grey and dark grey shading represent 80% and 95% credible intervals, respectively. Data points are also plotted.

consistent with them being large populations and important sources of natural recruitment (Gilbert and Whitlock 2015; Waples et al. 2016; Zampatti et al. 2019, 2021). The apparently large effective population sizes of the lower reaches of the Murray and Darling rivers may be contributed to by natural recruitment from multiple upstream reaches and tributaries (and also downstream reaches in the case of the Darling River; Stuart and Sharpe 2020; Zampatti et al. 2021; Thiem et al. 2023). Relatively low levels of kin-associated population genetic substructure in the Darling River (LDA, UDA), which is subject to some stocking (Table 1), likely reflect a buffering effect of large effective population size against drift (e.g., Rougemont et al. 2019) and/or a lower retention of stocked fish due to strong natural recruitment, which can occur under some conditions in the Darling River (Thiem et al. 2022).

### 4.3 | Genetic Consequences of Stocking

Genetic evidence of kin-associated population genetic substructure in wild-caught samples was widespread across the MDB and has increased over time (1991–2016, based on the age range of individuals sampled). Increasing prevalence of stocked individuals through time is consistent with increasingly larger numbers of fish stocked as part of large-scale hatchery breeding programmes (Hunt and Jones 2018; Table 1). Extended periods of reduced natural recruitment in some areas during the Millennium drought (2001–2009) may also have contributed to increasing genetic evidence of stocking post-2000 (van Dijk et al. 2013; Zampatti and Leigh 2013). Basin-wide estimates of effective population size for specific binned cohorts of golden perch were consistently orders of magnitude lower for stocked compared to equivalent wild origin individuals (stocked per binned cohort $N_e$ estimates ranged 63–156; wild per binned cohort *N*e estimates ranged 494–∞). Although these basin-wide estimates are unlikely to reflect the true effective population size of the total MDB population (owing to relatively small sample sizes and pooling of samples across large geographic areas), they highlight the comparatively small effective population size of the stocked population relative to the wild metapopulation.

We detected genetic evidence of stocked kin in major rivers that still have some potential for natural recruitment and/or are considered part of the connected metapopulation for management purposes, which raises concerns for the genetic diversity of persisting wild populations. For example, stocking can lead to genetic erosion through genetic swamping and associated declines in effective population size, even with an increase in census population size (Laikre et al. 2010; Christie et al. 2012; Almodóvar et al. 2020; Hagen et al. 2020; Klütsch et al. 2021; McMillan et al. 2023; May et al. 2024). Because genetic diversity is a critical resource by which populations adapt and respond to environmental change, its erosion may risk compromising the long-term sustainability and resilience of wild golden perch (Frankham et al. 2019).

Data collected did not allow us to test explicitly for evidence of stocked individuals breeding and the relative genetic contributions of stocked versus wild origin fish to spawning and recruitment. Captive-bred fish are often reported as experiencing reduced fitness in the wild compared with their wild conspecifics (Christie et al. 2014; Bouchard et al. 2022). However, even if the contribution to recruitment events by stocked fish is low, the presence of large numbers of stocked fish may create more competition for space and resources, potentially leading to poor

survival and recruitment of wild fish. Alternatively, if stocked individuals dominate in wild populations and make large contributions to natural recruitment events, it is possible that parts of the MDB may become vulnerable to negative inbreeding effects due to the presence of many close kin. These inbreeding effects can include reduced survival and fitness of offspring and reduced size of adult fish (Crnokrak and Roff 1999; Naish et al. 2013; Hedrick and Garcia-Dorado 2016). Future studies exploring the genetic contributions of stocked fish to spawning and recruitment in the wild, for instance by sampling larval recruits, will support a stronger understanding of the consequences of stocking for persisting wild golden perch in areas where natural recruitment still occurs.

### 4.4 | Detection of Highly Heterozygous Fish

Highly heterozygous fish were detected in the study, including a number that were nearly twice the average individual heterozygosity value. Although signatures of hybridisation among golden perch lineages from different basins (either natural or inadvertent due to stocking) have been reported elsewhere (Attard et al. 2022), the majority of highly heterozygous fish in our study did not appear to be of hybrid origin. At least half of the highly heterozygous fish appeared to be wild in origin, and heterozygous individuals were detected across nine different rivers, but almost half (14/33) were detected in the GIN and GWY rivers. It is possible that high heterozygosity values for these individuals may represent some type of sequencing artefact or triploidy (although we are not aware of any known cases of triploidy in golden perch).

### 4.5 | Scope to Adapt Fisheries Management Practices to Enhance Genetic Diversity

Many hatcheries in the MBD have guidelines for protecting the genetic integrity of wild populations (Rowland and Tully 2004; Hunt and Jones 2018). However, our findings suggest there are opportunities to build on current fisheries management practices to promote the genetic diversity of stocked fish and improve the effectiveness of stocking and the long-term sustainability of wild golden perch. From a practical perspective, the effective population size of stocked fish may be improved through a range of measures including increasing the number of broodstock used per consignment of stocked individuals to be released, increasing rates of broodstock rotation both within and among hatcheries and sourcing broodstock from diverse source populations. Genetic information can also be used to limit inbreeding among captive broodstock and equalise family contributions of broodstock to increase the effective population size of fish being stocked (Pregler et al. 2024). Captive breeding software exists that can help guide the selection of mate pairs in a way that minimises relatedness and reduces family structure and over-representation of close kin (e.g., PMx, SWINGER Lacy et al. 2012; Sandoval-Castillo et al. 2017).

With such a high prevalence of stocked close kin in some areas of the MDB, there is a high risk of collecting previously stocked fish as hatchery broodstock if broodstock collection overlaps spatially with liberation sites. This could lead to unintended pairing of related stocked individuals in hatcheries and compromised fitness of offspring. To minimise the chance of collecting previously stocked fish as broodstock, populations with large effective population sizes should be prioritised as sources for broodstock as they harbour the greatest genetic diversity and are less dominated by stocked fish. Strong candidate areas for sourcing broodstock are areas associated with high effective population size and minimal stocking impacts, such as the lower parts of the Murray–Darling system (LMU, LDA). A recent basin-wide programme, FishGen, has been developed to catalogue genotypes of hatchery broodstock and advise hatcheries annually of related fish in their programmes, allowing them to make informed choices to avoid inbreeding. Such programmes are important initiatives, and our results highlight the critical importance of screening the genetic makeup of new broodstock as they enter hatchery programmes to avoid pairing of related individuals and to check for previously stocked individuals.

To promote evolutionary resilience, specific genetic attributes of the broodstock and individuals being stocked may warrant consideration. For example, individuals may possess adaptive variants that may be beneficial under climate change (Booth et al. 2024) or that may be associated with key phenotypic traits (e.g., growth variation or dispersal propensity; Barrow, Yen, Koehn, and Morrongiello 2021; Barrow, Yen, Koehn, Zampatti, et al. 2021). There is also scope to explore the potential for cross-basin hybridisation to boost evolutionary potential in depauperate areas (Lutz et al. 2021; Brauer et al. 2023).

### 4.6 | Concluding Remarks

Understanding the genetic implications of stocking for species with high dispersal and genetic connectivity is challenging. By integrating genetic and otolith chemistry data at a system scale, we were able to make novel inferences about stocking that would not be possible utilising either data type alone. Most past studies that have used both genetic and otolith data have focused on salmonids and primarily on stock delineation, rather than on understanding the genetic implications of stocking for wild populations (Tanner et al. 2014; Taillebois et al. 2017, 2021). As such, our work provides a valuable and novel case study on combining genetic and otolith data types for a non-salmonid species.

As is the case for most stocking programmes worldwide, stocking of golden perch in the MDB has been strongly focused on the number of fish being released, with the primary motivation being to support recreational fisheries (Claussen and Philipp 2023). Evidence of kin-associated genetic substructure linked to stocking in interconnected parts of major river systems may pose a threat to the genetic diversity of persisting wild golden perch. Going forward, modifying breeding and stocking programmes to place a greater emphasis on the genetic diversity and effective population size of stocked fish, as well as continuing to implement management strategies that promote in situ recruitment and connectivity (e.g., habitat protection and restoration, removal of barriers, environmental water), is likely to

have positive outcomes for the evolutionary resilience of wild golden perch in the future.

## Author Contributions

Project conceptualisation and design: K.A.H., J.L., Z.T., B.P.Z., J.D.T., N.P.M.; Funding acquisition: K.A.H., J.L., J.R.M., J.D.L.Y., L.B.B.; Sample and data curation C.M.B., G.L.B., D.D., G.H., M.J.J., W.M.K., J.A.L., S.M.C.R., A.S., J.D.T., L.B.B., E.J.B., C.J.B.; Analysis of genetic data: K.A.H., J.O., J.D.L.Y., C.J.B.; Analysis of otolith data: K.K.-G., A.H., B.G.F.; Genome sequencing and assembly: P.K., E.L.A., O.D.; K.A.H. wrote the original draft and all authors contributed to subsequent drafts. All authors approved the final version of this manuscript for publication.


## Acknowledgements

This project was supported by ARC DECRA DE190100636. Additional funding was provided by La Trobe University, The University of Melbourne and the Goulburn Broken Catchment Management Authority. Golden Perch sampling across the MDB was funded by the MDBA, the CEWO LTIM and EWKR programmes and a range of State-based monitoring programmes in NSW, Queensland and Victoria, particularly the Victorian Environmental Flows Monitoring and Assessment Program. Field sampling teams included Kris Pitman, Justin O'Mahony; Andrew Pickworth, John Mahoney, Justin O'Mahony, Stephen Saddlier, Kim Loeun, Steven Brooks, Mark Babbs, Tom Butterfield, Jonathon Doyle, Duncan McLay, Rohan Rehwinkel, Chris Smith and Ian Wooden. The authors thanks Sarah Willington and Adrian Kitchingman for sample and database curation and Matthew McLellan for assistance in obtaining stocking records and feedback on earlier drafts. Work at the Molecular Ecology Lab at Flinders University was supported by ARC grants LP0667952, FT130101068 and DP190102533. The tissue sample for the Hi-C genome was provided by Narrandera Fisheries Centre (NSW Department of Primary Industries and Regional Development). The Hi-C work was supported by resources provided by DNA Zoo Australia, The University of Western Australia (UWA) and by Ashling Charles. E.L.A. was supported by the Welch Foundation (Q-1866), a McNair Medical Institute Scholar Award, an NIH Encyclopedia of DNA Elements Mapping Center Award (UM1HG009375), a US–Israel Binational Science Foundation Award (2019276), the Behavioural Plasticity Research Institute (NSF DBI-2021795), NSF Physics Frontiers Center Award (NSF PHY-2019745) and by the National Human Genome Research Institute of the National Institutes of Health (RM1HG011016-01A1). Computational support for the Hi-C genome was provided by the Pawsey Supercomputing Centre with funding from the Australian Government and the Government of Western Australia. DNA Zoo also acknowledges support from Illumina and IBM. We thank three anonymous reviewers for their valuable feedback on the manuscript. We acknowledge the Traditional Owners of the land on which we work and on which this research was conducted and pay our respects to their Elders, past and present. Open access publishing facilitated by La Trobe University, as part of the Wiley - La Trobe University agreement via the Council of Australian University Librarians.


## Disclosure

Benefit-Sharing Statement: This project is a national collaboration including government and non-government scientists from the country where the study system is based. Findings of this research have been shared with stakeholders and the broader scientific community. Benefits from this research accrue from the sharing of our data and results on public databases as described above.

## Conflicts of Interest

The authors declare no conflicts of interest.

## Data Availability Statement

Raw dart data will be uploaded to NCBI SRA. Bioinformatics code is available at: https://github.com/James-ODwyer/Golden_perch_Bioinformatics. The Geofabric Surface Network can be accessed from http://www.bom.gov.au/water/geofabric/. The chromosome-level genome assembly (GOP001_1.1_HiC) is freely available on the DNA Zoo website (https://www.dnazoo.org/assemblies/Macquaria_ambigua). The raw Hi-C data is available via SRA, accession number SRX12373228. Code for hybridisation tests is available at: https://github.com/pygmyperch/MDB_GP_hybrid_analyses. All other model codes, custom functions and data are available at: https://github.com/kharrisson/goldenperch.

## References


Alexander, D. H., J. Novembre, and K. Lange. 2009. "Fast Model-Based Estimation of Ancestry in Unrelated Individuals." *Genome Research* 19, no. 9: 1655–1664.

Almodóvar, A., S. Leal, G. G. Nicola, J. L. Hórreo, E. García-Vázquez, and B. Elvira. 2020. "Long-Term Stocking Practices Threaten the Original Genetic Diversity of the Southernmost European Populations of Atlantic Salmon *Salmo salar*." *Endangered Species Research* 41: 303–317.

Araki, H., B. Cooper, and M. S. Blouin. 2007. "Genetic Effects of Captive Breeding Cause a Rapid, Cumulative Fitness Decline in the Wild." *Science* 318: 100–103.

Araki, H., and C. Schmid. 2010. "Is Hatchery Stocking a Help or Harm? Evidence, Limitations and Future Directions in Ecological and Genetic Surveys." *Aquaculture* 308: S2–S11.

Attard, C. R. M., C. J. Brauer, J. Sandoval-Castillo, et al. 2018. "Ecological Disturbance Influences Adaptive Divergence Despite High Gene Flow in Golden Perch (*Macquaria ambigua*): Implications for Management and Resilience to Climate Change." *Molecular Ecology* 27, no. 1: 196–215.

Attard, C. R. M., J. Sandoval-Castillo, D. M. Gilligan, P. J. Unmack, L. K. Faulks, and L. B. Beheregaray. 2022. "Genomics Outperforms Genetics to Manage Mistakes in Fisheries Stocking of Threatened Species." *Biodiversity and Conservation* 31, no. 3: 895–908.

Bailey, R. I. 2024. "Bayesian Hybrid Index and Genomic Cline Estimation With the R Package." *Molecular Ecology Resources* 24, no. 2: e13910.

Balcombe, S. R., A. A. Arthington, N. D. Foster, M. C. Thoms, G. G. Wilson, and S. E. Bunn. 2006. "Fish Assemblages of an Australian Dryland River: Abundance, Assemblage Structure and Recruitment Patterns in the Warrego River, Murray-Darling Basin." *Marine and Freshwater Research* 57, no. 6: 619–633.

Barrow, J. S., J. D. L. Yen, J. D. Koehn, and J. R. Morrongiello. 2021. "Increasing Early Life Growth of Hatchery-Reared Freshwater Fish Can Improve Stocking Outcomes." *Marine and Freshwater Research* 72, no. 4: 526–533.

Barrow, J. S., J. D. L. Yen, J. D. Koehn, et al. 2021. "Lifetime Movement History Is Associated With Variable Growth of a Potamodromous Freshwater Fish." *Journal of Animal Ecology* 90, no. 11: 2560–2572.

Beheregaray, L. B., L. V. Pfeiffer, C. R. M. Attard, et al. 2017. "Genome-Wide Data Delimits Multiple Climate-Determined Species Ranges in a Widespread Australian Fish, the Golden Perch (*Macquaria ambigua*)." *Molecular Phylogenetics and Evolution* 111: 65–75.

Bertram, A., J. Bell, C. Brauer, et al. 2024. "Estimation of Effective Number of Breeders and Effective Population Size in an Abundant and Heavily Exploited Marine Teleost." *Evolutionary Applications* 17, no. 7: e13758.

Booth, E. J., C. Brauer, J. Sandoval-Castillo, et al. 2024. "Genomic Vulnerability to Climate Change of an Australian Migratory Freshwater

Fish, the Golden Perch (*Macquaria ambigua*)." *Molecular Ecology* 33: e17570.

Bootsma, M. L., L. Miller, G. G. Sass, P. T. Euclide, and W. A. Larson. 2021. "The Ghosts of Propagation Past: Haplotype Information Clarifies the Relative Influence of Stocking History and Phylogeographic Processes on Contemporary Population Structure of Walleye (*Sander vitreus*)." *Evolutionary Applications* 14, no. 4: 1124–1144.

Bouchard, R., K. Wellband, L. Lecomte, L. Bernatchez, and J. April. 2022. "Effects of Stocking at the Parr Stage on the Reproductive Fitness and Genetic Diversity of a Wild Population of Atlantic Salmon (*Salmo salar* L.)." *Evolutionary Applications* 15, no. 5: 838–852.

Brauer, C. J., J. Sandoval-Castillo, K. Gates, et al. 2023. "Natural Hybridization Reduces Vulnerability to Climate Change." *Nature Climate Change* 13, no. 3: 282–289.

Buckley, S. J., C. Brauer, C. Lamin, P. Rose, D. E. Vornicu, and L. B. Beheregaray. 2024. "A Community-Driven Captive-Breeding and Reintroduction Program Maintains Genetic Diversity in a Threatened Freshwater Fish." *Conservation Science and Practice* 6, no. 1: e13054.

Buerkle, C. A. 2005. "Maximum-Likelihood Estimation of a Hybrid Index Based on Molecular Markers." *Molecular Ecology Notes* 5, no. 3: 684–687.

Butler, G. L., L. M. Cameron, D. W. Coleman, B. C. Ebner, J. D. Thiem, and L. Carpenter-Bundhoo. 2024. "Risk v. Reward for Responsive Movements by a Highly Mobile Fish Species in a Flow-Constrained and Barrier-Laden River." *Marine and Freshwater Research* 75, no. 15: MF24053.

Christie, M. R., M. J. Ford, and M. S. Blouin. 2014. "On the Reproductive Success of Early-Generation Hatchery Fish in the Wild." *Evolutionary Applications* 7, no. 8: 883–896.

Christie, M. R., M. L. Marine, R. A. French, R. S. Waples, and M. S. Blouin. 2012. "Effective Size of a Wild Salmonid Population Is Greatly Reduced by Hatchery Supplementation." *Heredity* 109, no. 4: 254–260.

Claussen, J. E., and D. P. Philipp. 2023. "Assessing the Role of Supplementation Stocking: A Perspective." *Fisheries Management and Ecology* 30, no. 6: 583–591.

Coulon, A. 2010. "GENHET: An Easy-To-Use R Function to Estimate Individual Heterozygosity." *Molecular Ecology Resources* 10, no. 1: 167–169.

Cowx, I. G. 1994. "Stocking strategies." *Fisheries Management and Ecology* 1, no. 1: 15–30.

Crnokrak, P., and D. A. Roff. 1999. "Inbreeding Depression in the Wild." *Heredity* 83, no. 3: 260–270.

Crook, D. A., D. J. O'Mahony, B. M. Gillanders, et al. 2016. "Contribution of Stocked Fish to Riverine Populations of Golden Perch (*Macquaria ambigua*) in the Murray-Darling Basin, Australia." *Marine and Freshwater Research* 67, no. 10: 1401–1409.

Crook, D. A., D. J. O'Mahony, A. C. Sanger, A. R. Munro, B. M. Gillanders, and S. Thurstan. 2009. "Development and Evaluation of Methods for Osmotic Induction Marking of Golden Perch With Calcein and Alizarin Red S." *North American Journal of Fisheries Management* 29, no. 2: 279–287.

Crook, D. A., H. T. Schilling, D. M. Gilligan, et al. 2023. "Multi-Decadal Trends in Large-Bodied Fish Populations in the New South Wales Murray-Darling Basin, Australia." *Marine and Freshwater Research* 74, no. 11: 899–916.

Curtis, J. M., G. W. Stunz, R. D. Overath, and R. R. Vega. 2014. "Otolith Chemistry Can Discriminate Signatures of Hatchery-Reared and Wild Spotted Seatrout." *Fisheries Research* 153: 31–40.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. "NEESTIMATOR v2: Re-Implementation of Software for the Estimation of Contemporary Effective Population Size (N-e) From Genetic Data." *Molecular Ecology Resources* 14, no. 1: 209–214.

Dudchenko, O., S. S. Batra, A. D. Omer, et al. 2017. "De Novo Assembly of the *Aedes aegypti* Genome Using Hi-C Yields Chromosome-Length Scaffolds." *Science* 356, no. 6333: 92–95.

Dudchenko, O., M. S. Shamim, S. S. Batra, et al. 2018. "The Juicebox Assembly Tools Module Facilitates de Novo Assembly of Mammalian Genomes With Chromosome-Length Scaffolds for Under $1000." *BioRxiv*, 254797.

Earl, J., B. Ingram, T. Hart, and D. Crook. 2023. "Golden Perch *Macquaria ambigua*." In *2024, Status of Australian Fish Stocks Reports 2024*, edited by A. Roelofs, T. Piddocke, C. Ashby, et al. Fisheries Research and Development Corporation.

Evanno, G., S. Regnaut, and J. Goudet. 2005. "Detecting the Number of Clusters of Individuals Using the Software STRUCTURE: A Simulation Study." *Molecular Ecology* 14, no. 8: 2611–2620.

Falush, D., M. Stephens, and J. K. Pritchard. 2003. "Inference of Population Structure Using Multilocus Genotype Data: Linked Loci and Correlated Allele Frequencies." *Genetics* 164, no. 4: 1567–1587.

Faulks, L. K., D. M. Gilligan, and L. B. Beheregaray. 2010. "Islands of Water in a Sea of Dry Land: Hydrological Regime Predicts Genetic Diversity and Dispersal in a Widespread Fish From Australia's Arid Zone, the Golden Perch (*Macquaria ambigua*)." *Molecular Ecology* 19, no. 21: 4723–4737.

Ferchaud, A. L., M. Laporte, C. Perrier, and L. Bernatchez. 2018. "Impact of Supplementation on Deleterious Mutation Distribution in an Exploited Salmonid." *Evolutionary Applications* 11, no. 7: 1053–1065.

Flitcroft, R., M. S. Cooperman, I. J. Harrison, D. Juffe-Bignoli, and P. J. Boon. 2019. "Theory and Practice to Conserve Freshwater Biodiversity in the Anthropocene." *Aquatic Conservation-Marine and Freshwater Ecosystems* 29, no. 7: 1013–1021.

Forbes, J., R. J. Watts, W. A. Robinson, et al. 2016. "Assessment of Stocking Effectiveness for Murray Cod (*Maccullochella peelii*) and Golden Perch (*Macquaria ambigua*) in Rivers and Impoundments of South-Eastern Australia." *Marine and Freshwater Research* 67, no. 10: 1410–1419.

Francis, R. M. 2017. "POPHELPER: An R Package and Web App to Analyse and Visualize Population Structure." *Molecular Ecology Resources* 17, no. 1: 27–32.

Frankham, R., J. D. Ballou, K. Ralls, et al. 2019. *A Practical Guide for Genetic Management of Fragmented Animal and Plant Populations Introduction*. Oxford University Press.

Frankham, R., C. J. A. Bradshaw, and B. W. Brook. 2014. "Genetics in Conservation Management: Revised Recommendations for the 50/500 Rules, Red List Criteria and Population Viability Analyses." *Biological Conservation* 170: 56–63.

Furlan, E. M., L. J. Baumgartner, M. Duncan, et al. 2024. "Swinging Back From the Brink? Polygamous Mating Strategies Revealed for an Iconic Threatened Freshwater Fish." *Science of the Total Environment* 919: 170808.

Gilbert, K. J., and M. C. Whitlock. 2015. "Evaluating Methods for Estimating Local Effective Population Size With and Without Migration." *Evolution* 69, no. 8: 2154–2166.

Gompert, Z., and C. A. Buerkle. 2010. "INTROGRESS: A Software Package for Mapping Components of Isolation in Hybrids." *Molecular Ecology Resources* 10, no. 2: 378–384.

Goodrich, B., J. Gabry, I. Ali, and S. Brilleman. 2020. "rstanarm: Bayesian Applied Regression Modeling via Stan." R Package Version 2.1.

Gossieaux, P., L. Bernatchez, P. Siroi, and D. Garant. 2019. "Impacts of Stocking and Its Intensity on Effective Population Size in Brook Charr (*Salvelinus fontinalis*) Populations." *Conservation Genetics* 20, no. 4: 729–742.

Goudet, J. 2005. "HIERFSTAT, a Package for R to Compute and Test Hierarchical F-Statistics." *Molecular Ecology Notes* 5, no. 1: 184–186.

Hagen, I. J., O. Ugedal, A. J. Jensen, et al. 2020. "Evaluation of Genetic Effects on Wild Salmon Populations From Stock Enhancement." *ICES Journal of Marine Science* 78, no. 3: 900–909.

Hansen, M. M., D. J. Fraser, K. Meier, and K. L. D. Mensberg. 2009. "Sixty Years of Anthropogenic Pressure: A Spatio-Temporal Genetic Analysis of Brown Trout Populations Subject to Stocking and Population Declines." *Molecular Ecology* 18, no. 12: 2549–2562.

Hare, M. P., L. Nunney, M. K. Schwartz, et al. 2011. "Understanding and Estimating Effective Population Size for Practical Application in Marine Species Management." *Conservation Biology* 25, no. 3: 438–449.

Hedrick, P. W., and A. Garcia-Dorado. 2016. "Understanding Inbreeding Depression, Purging, and Genetic Rescue." *Trends in Ecology and Evolution* 31, no. 12: 940–952.

Hunt, T. L., M. S. Allen, J. Douglas, and A. Gason. 2010. "Evaluation of a Sport Fish Stocking Program in Lakes of the Southern Murray-Darling Basin, Australia." *North American Journal of Fisheries Management* 30, no. 3: 805–811.

Hunt, T. L., and P. Jones. 2018. "Informing the Great Fish Stocking Debate: An Australian Case Study." *Reviews in Fisheries Science and Aquaculture* 26, no. 3: 275–308.

Janowitz-Koch, I., C. Rabe, R. Kinzer, D. Nelson, M. A. Hess, and S. R. Narum. 2019. "Long-Term Evaluation of Fitness and Demographic Effects of a Chinook Salmon Supplementation Program." *Evolutionary Applications* 12, no. 3: 456–469.

Jones, O. R., and J. L. Wang. 2010. "COLONY: A Program for Parentage and Sibship Inference From Multilocus Genotype Data." *Molecular Ecology Resources* 10, no. 3: 551–555.

Kardos, M., G. Luikart, and F. W. Allendorf. 2015. "Measuring Individual Inbreeding in the Age of Genomics: Marker-Based Measures Are Better Than Pedigrees." *Heredity* 115, no. 1: 63–72.

King, A. J., D. C. Gwinn, Z. Tonkin, J. Mahoney, S. Raymond, and L. Beesley. 2016. "Using Abiotic Drivers of Fish Spawning to Inform Environmental Flow Management." *Journal of Applied Ecology* 53, no. 1: 34–43.

Klütsch, C. F. C., S. N. Maduna, N. Polikarpova, et al. 2021. "Temporal Analysis Shows Relaxed Genetic Erosion Following Improved Stocking Practices in a Subarctic Transnational Brown Trout Population." *Scientific Reports* 11, no. 1: 17396.

Koehn, J., and W. O'Connor. 1990. "Biological information for management of native freshwater fish in Victoria, Department of Conservation and Environment, Freshwater Fish Management Branch, Arthur Rylah Institute for Environmental Research."

Koehn, J. D., S. M. Raymond, I. Stuart, et al. 2020. "A Compendium of Ecological Knowledge for Restoration of Freshwater Fishes in Australia's Murray-Darling Basin." *Marine and Freshwater Research* 71, no. 11: 1391–1463.

Kopelman, N. M., J. Mayzel, M. Jakobsson, N. A. Rosenberg, and I. Mayrose. 2015. "Clumpak: A Program for Identifying Clustering Modes and Packaging Population Structure Inferences Across K." *Molecular Ecology Resources* 15, no. 5: 1179–1191.

Koster, W. M., D. R. Dawson, C. Liu, P. D. Moloney, D. A. Crook, and J. R. Thomson. 2017. "Influence of Streamflow on Spawning-Related Movements of Golden Perch in South-Eastern Australia." *Journal of Fish Biology* 90, no. 1: 93–108.

Lacy, R. C., J. D. Ballou, and J. P. Pollak. 2012. "PMx: Software Package for Demographic and Genetic Analysis and Management of Pedigreed Populations." *Methods in Ecology and Evolution* 3, no. 2: 433–437.

Laikre, L., M. K. Schwartz, R. S. Waples, N. Ryman, and G. W. Grp. 2010. "Compromising Genetic Diversity in the Wild: Unmonitored Large-Scale Release of Plants and Animals." *Trends in Ecology and Evolution* 25, no. 9: 520–529.

Le Cam, S., C. Perrier, A. L. Besnard, L. Bernatchez, and G. Evanno. 2015. "Genetic and Phenotypic Changes in an Atlantic Salmon Population Supplemented With Non-local Individuals: A Longitudinal Study Over 21 Years." *Proceedings of the Royal Society B-Biological Sciences* 282, no. 1802: 20142765.

Liermann, M., G. Pess, M. McHenry, et al. 2017. "Relocation and Recolonization of Coho Salmon in Two Tributaries to the Elwha River: Implications for Management and Monitoring." *Transactions of the American Fisheries Society* 146, no. 5: 955–966.

Lutz, M. L., Z. Tonkin, J. D. L. Yen, et al. 2021. "Using Multiple Sources During Reintroduction of a Locally Extinct Population Benefits Survival and Reproduction of an Endangered Freshwater Fish." *Evolutionary Applications* 14, no. 4: 950–964.

Lyon, J. P., C. Todd, S. J. Nicol, et al. 2012. "Reintroduction Success of Threatened Australian Trout Cod (*Maccullochella macquariensis*) Based on Growth and Reproduction." *Marine and Freshwater Research* 63, no. 7: 598–605.

Mallen-Cooper, M., and I. G. Stuart. 2003. "Age, Growth and Non-flood Recruitment of Two Potamodromous Fishes in a Large Semi-Arid/Temperate River System." *River Research and Applications* 19, no. 7: 697–719.

Marie, A. D., L. Bernatchez, and D. Garant. 2010. "Loss of Genetic Integrity Correlates With Stocking Intensity in Brook Charr (*Salvelinus fontinalis*)." *Molecular Ecology* 19, no. 10: 2025–2037.

Marshall, I. R., C. J. Brauer, S. D. Wedderburn, et al. 2022. "Longitudinal Monitoring of Neutral and Adaptive Genomic Diversity in a Reintroduction." *Conservation Biology* 36, no. 4: e13889.

May, S. A., K. R. Shedd, K. M. Gruenthal, et al. 2024. "Salmon Hatchery Strays Can Demographically Boost Wild Populations at the Cost of Diversity: Quantitative Genetic Modelling of Alaska Pink Salmon." *Royal Society Open Science* 11, no. 7: 240455.

McMillan, J. R., B. Morrison, N. Chambers, et al. 2023. "A Global Synthesis of Peer-Reviewed Research on the Effects of Hatchery Salmonids on Wild Salmonids." *Fisheries Management and Ecology* 30, no. 5: 446–463.

Nadachowska-Brzyska, K., L. Dutoit, L. Smeds, M. Kardos, L. Gustafsson, and H. Ellegren. 2021. "Genomic Inference of Contemporary Effective Population Size in a Large Island Population of Collared Flycatchers (*Ficedula albicollis*)." *Molecular Ecology* 30, no. 16: 3965–3973.

Naish, K. A., T. R. Seamons, M. B. Dauer, L. Hauser, and T. P. Quinn. 2013. "Relationship Between Effective Population Size, Inbreeding and Adult Fitness-Related Traits in a Steelhead (*Oncorhynchus mykiss*) Population Released in the Wild." *Molecular Ecology* 22, no. 5: 1295–1309.

Naish, K. A., J. E. Taylor III, P. S. Levin, et al. 2007. "An Evaluation of the Effects of Conservation and Fishery Enhancement Hatcheries on Wild Populations of Salmon." *Advances in Marine Biology* 53: 61–194.

O'Sullivan, R. J., T. Aykanat, S. Johnston, et al. 2021. "Captive-Bred Atlantic Salmon Released Into the Wild Have Fewer Offspring Than Wild-Bred Fish and Decrease Population Productivity." *Proceedings of the Royal Society B-Biological Sciences* 288: 20201671.

Pavlova, A., K. A. Harrisson, R. Turakulov, et al. 2022. "Labile Sex Chromosomes in the Australian Freshwater Fish Family Percichthyidae." *Molecular Ecology Resources* 22, no. 4: 1639–1655.

Pavlova, A., N. M. Schneller, M. Lintermans, M. Beitzel, D. A. Robledo-Ruiz, and P. Sunnucks. 2024. "Planning and Implementing Genetic Rescue of an Endangered Freshwater Fish Population in a Regulated River, Where Low Flow Reduces Breeding Opportunities and May

Trigger Inbreeding Depression." *Evolutionary Applications* 17, no. 4: e13679.

Pregler, K. C., A. Clemento, M. Grill, P. Adelizi, S. M. Carlson, and J. C. Garza. 2024. "Reintroduction of Spring-Run Chinook Salmon in the San Joaquin River: Evaluating Genetic and Phenotypic Effects of Captive Breeding." *Conservation Science and Practice* 6, no. 9: e13176.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. "Inference of Population Structure Using Multilocus Genotype Data." *Genetics* 155, no. 2: 945–959.

R Core Team. 2018. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing.

Radinger, J., S. Matern, T. Klefoth, et al. 2023. "Ecosystem-Based Management Outperforms Species-Focused Stocking for Enhancing Fish Populations." *Science* 379, no. 6635: 946–951.

Ramasamy, R. K., S. Ramasamy, B. B. Bindroo, and V. G. Naik. 2014. "STRUCTURE PLOT: A Program for Drawing Elegant STRUCTURE Bar Plots in User Friendly Interface." *Springerplus* 3: 431.

Rao, S. S. P., M. H. Huntley, N. C. Durand, et al. 2014. "A 3D Map of the Human Genome at Kilobase Resolution Reveals Principles of Chromatin Looping." *Cell* 159, no. 7: 1665–1680.

Reid, A. J., A. K. Carlson, I. F. Creed, et al. 2019. "Emerging Threats and Persistent Conservation Challenges for Freshwater Biodiversity." *Biological Reviews* 94, no. 3: 849–873.

Reis-Santos, P., B. M. Gillanders, A. M. Sturrock, et al. 2023. "Reading the Biomineralized Book of Life: Expanding Otolith Biogeochemical Research and Applications for Fisheries and Ecosystem-Based Management." *Reviews in Fish Biology and Fisheries* 33, no. 2: 451–452.

Reynolds, L. 1983. "Migration Patterns of Five Fish Species in the Murray-Darling River System." *Marine and Freshwater Research* 34, no. 6: 857–871.

Rougemont, Q., A. Carrier, J. Le Luyer, et al. 2019. "Combining Population Genomics and Forward Simulations to Investigate Stocking Impacts: A Case Study of Muskellunge (*Esox masquinongy*) From the St. Lawrence River Basin." *Evolutionary Applications* 12, no. 5: 902–922.

Rowland, S. 2013. "Hatchery Production for Conservation and Stock Enhancement: The Case of Australian Freshwater Fish." In *Advances in Aquaculture Hatchery Technology*, 557–595. Woodhead Publishing.

Rowland, S. J., and P. Tully. 2004. *Hatchery Quality Assurance Program for Murray Cod (Madcullochella Peelii Peelii), golden Perch (Macquaria ambigua) and Silver Perch (Bidyanus bidyanus)*. NSW Department if Primary Industries.

Ryman, N., and L. Laikre. 1991. "Effects of Supportive Breeding on the Genetically Effective Population-Size." *Conservation Biology* 5, no. 3: 325–329.

Sandoval-Castillo, J., C. R. M. Attard, S. Marri, C. J. Brauer, L. M. Möller, and L. B. Beheregaray. 2017. "SWINGER: A User-Friendly Computer Program to Establish Captive Breeding Groups That Minimize Relatedness Without Pedigree Information." *Molecular Ecology Resources* 17, no. 2: 278–287.

Shams, F., F. Dyer, R. Thompson, et al. 2019. "Karyotypes and Sex Chromosomes in Two Australian Native Freshwater Fishes, Golden Perch (*Macquaria ambigua*) and Murray Cod (*Maccullochella peelii*) (Percichthyidae)." *International Journal of Molecular Sciences* 20, no. 17: 4244.

Shedd, K. R., E. A. Lescak, C. Habicht, et al. 2022. "Reduced Relative Fitness in Hatchery-Origin Pink Salmon in Two Streams in Prince William Sound, Alaska." *Evolutionary Applications* 15, no. 3: 429–446.

Steele, C. A., M. Hess, S. Narum, and M. Campbell. 2019. "Parentage-Based Tagging: Reviewing the Implementation of a New Tool for an Old Problem." *Fisheries* 44: 412–422.

Stuart, I. G., and C. P. Sharpe. 2020. "Riverine Spawning, Long Distance Larval Drift, and Floodplain Recruitment of a Pelagophilic Fish: A Case Study of Golden Perch (*Macquaria ambigua*) in the Arid Darling River, Australia." *Aquatic Conservation-Marine and Freshwater Ecosystems* 30, no. 4: 675–690.

Taillebois, L., D. P. Barton, D. A. Crook, et al. 2017. "Strong Population Structure Deduced From Genetics, Otolith Chemistry and Parasite Abundances Explains Vulnerability to Localized Fishery Collapse in a Large Sciaenid Fish, *Protonibea diacanthus*." *Evolutionary Applications* 10, no. 10: 978–993.

Taillebois, L., D. Davenport, D. P. Barton, et al. 2021. "Integrated Analyses of SNP-Genotype and Environmental Data in a Continuously Distributed Snapper Species (*Lutjanus johnii*, Bloch, 1792) Reveals a Mosaic of Populations and a Challenge for Sustainable Management." *ICES Journal of Marine Science* 78, no. 9: 3212–3229.

Tanner, S. E., M. Perez, P. Presa, S. R. Thorrold, and H. N. Cabral. 2014. "Integrating Microsatellite DNA Markers and Otolith Geochemistry to Assess Population Structure of European Hake (*Merluccius merluccius*)." *Estuarine Coastal and Shelf Science* 142: 68–75.

Thériault, V., G. R. Moyer, L. S. Jackson, M. S. Blouin, and M. A. Banks. 2011. "Reduced Reproductive Success of Hatchery Coho Salmon in the Wild: Insights Into Most Likely Mechanisms." *Molecular Ecology* 20, no. 9: 1860–1869.

Thiem, J. D., L. J. Baumgartner, B. Fanson, A. Sadekov, Z. Tonkin, and B. P. Zampatti. 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, no. 2: 237–246.

Thiem, J. D., L. E. Michie, G. L. Butler, et al. 2023. "A Protected Flow Breaks the Drought for Golden Perch (*Macquaria ambigua*) Spawning Along an Extensive Semi-Arid River System." *Ecohydrology* 16, no. 7: e2576.

Thiem, J. D., I. J. Wooden, L. J. Baumgartner, G. L. Butler, J. P. Forbes, and J. Conallin. 2017. "Recovery From a Fish Kill in a Semi-Arid Australian River: Can Stocking Augment Natural Recruitment Processes?" *Austral Ecology* 42, no. 2: 218–226.

Uglem, I., T. S. Kristiansen, C. M. Mejdell, D. Basic, and S. Mortensen. 2020. "Evaluation of Large-Scale Marking Methods in Farmed Salmonids for Tracing Purposes: Impact on Fish Welfare." *Reviews in Aquaculture* 12, no. 2: 600–625.

Utter, F. 2004. "Population Genetics, Conservation and Evolution in Salmonids and Other Widely Cultured Fishes: Some Perspectives Over Six Decades." *Journal of Fish Biology* 65: 323–324.

van der Meer, L., L. Abad, A. Gilardi, and R. Lovelace. 2023. "sfnetworks: Tidy Geospatial Networks."

van Dijk, A. I. J. M., H. E. Beck, R. S. Crosbie, et al. 2013. "The Millennium Drought in Southeast Australia (2001–2009): Natural and Human Causes and Implications for Water Resources, Ecosystems, Economy, and Society." *Water Resources Research* 49, no. 2: 1040–1057.

Waples, R., W. Larson, and R. Waples. 2016. "Estimating Contemporary Effective Population Size in Non-model Species Using Linkage Disequilibrium Across Thousands of Loci." *Heredity* 117: 233–240.

Waples, R. S., T. Antao, and G. Luikart. 2014. "Effects of Overlapping Generations on Linkage Disequilibrium Estimates of Effective Population Size." *Genetics* 197, no. 2: 603–769.

Welcomme, R. L., and D. M. Bartley. 1998. "Current Approaches to the Enhancement of Fisheries." *Fisheries Management and Ecology* 5, no. 5: 351–382.

Wringe, B. F., R. R. E. Stanley, N. W. Jeffery, E. C. Anderson, and I. R. Bradbury. 2017. "Hybriddetective: A Workflow and Package to Facilitate the Detection of Hybridization Using Genomic Data in r." *Molecular Ecology Resources* 17, no. 6: e275–e284.

Zampatti, B., and S. Leigh. 2013. "Effects of Flooding on Recruitment and Abundance of Golden Perch (*Macquaria ambigua* Ambigua) in the

Lower River Murray." *Ecological Management and Restoration* 14, no. 2: 135–143.

Zampatti, B. P. 2019. "Ecology and Population Dynamics of Golden Perch in a Fragmented, Flow-Impacted River: Implications for Conservation and Management." Ph.D. thesis. University of Adelaide.

Zampatti, B. P., B. G. Fanson, L. J. Baumgartner, et al. 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, no. 2: 223–236.

Zampatti, B. P., B. G. Fanson, A. Strawbridge, et al. 2019. "Basin-Scale Population Dynamics of Golden Perch and Murray Cod: Relating Flow to Provenance, Movement and Recruitment in the Murray–Darling Basin." Murray–Darling Basin Environmental Water Knowledge and Research Project—Fish Theme Research Report. A. Price, S. Balcombe, P. Humphries, A. King and B. P. Zampatti, La Trobe University. CFE Publication 223. Commonwealth Environmental Water Office. https://opal.latrobe.edu.au/articles/journal_contribution/Murray-Darling_Basin_Environmental_Water_Knowledge_and_Research_Project_Fish_Theme_Research_Report/8942147.

Zampatti, B. P., S. J. Leigh, C. M. Bice, and P. J. Rogers. 2018. "Multiscale Movements of Golden Perch (Percichthyidae: *Macquaria ambigua*) in the River Murray, Australia." *Austral Ecology* 43: 763–774.

Zampatti, B. P., S. J. Leigh, P. J. Wilson, et al. 2021. "Otolith Chemistry Delineates the Influence of Natal Origin, Dispersal and Flow on the Population Dynamics of Golden Perch (*Macquaria ambigua*) in a Regulated River." *Marine and Freshwater Research* 72, no. 10: 1484–1495.

## Supporting Information

Additional supporting information can be found online in the Supporting Information section.