Inland Waters &
Catchment Ecology

Chowilla Icon Site Fish Intervention Monitoring 2021/22

C. M. Bice[1], T.H. D'Ombrain[2], J. F. Fredberg[1], K. A. Harrisson[2], N. P. Murphy[2], J. O'Dwyer[3] and B. P. Zampatti[4]

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December 2023

Report to the Department for Environment and Water

Government of South Australia
Department of Primary Industries and Regions

Government of South Australia
Department for Environment and Water

SARDI
SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE

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EXECUTIVE SUMMARY

The Chowilla Anabranch and Floodplain system (hereafter Chowilla) comprises the largest remaining area of undeveloped floodplain habitat in the lower River Murray, encompassing a series of anabranching creeks, backwaters, wetlands and terminal lakes that bypass Lock and Weir No. 6 (hereafter Lock 6) on the River Murray. Because of this, Chowilla provides unique structural and hydraulic habitats that maintain remnant populations of endangered riverine fauna, including Murray cod (*Maccullochella peelii*). Nonetheless, the ecological communities of both aquatic and floodplain environments are considered degraded due to the impacts of river regulation – primarily fragmentation by barriers to flow and fish passage, and decreased flooding frequency – and the site is now the focus of a range of interventions to promote ecological rehabilitation.

In 2014, the Chowilla Creek regulator and ancillary structures were constructed with the objective of using large-scale engineered floodplain inundation to maintain or improve 'ecological condition' of the floodplain. Operation of the regulator, however, poses several ecological risks, including: 1) fragmentation of habitats and obstruction of fish movement; and 2) alteration of stream hydraulics (e.g. reduced water velocities) that may influence the habitats of lotic fishes (e.g. Murray cod). These impacts may affect Murray cod movement, spawning and recruitment, and ultimately, population dynamics. To better understand the response of Murray cod and other fish species to regulator operation and a range of other management interventions at Chowilla, an *intervention monitoring* program supports targeted investigations to inform adaptive management.

Intervention Monitoring in 2021/22 was comprised of three allied investigations. The primary objectives of these investigations were to:

1) Capture Murray cod larvae and young-of-year (YOY) derived from spawning in 2021 and, together with historic samples, undertake genetic analyses using kinship approaches to provide an estimate of reproductive variance, movement and breeding population size for comparison with previous and future spawning seasons;
2) Collect tissue samples from Murray cod captured during annual condition monitoring and undertake epigenetic analyses to estimate population age structure;
3) Monitor the movement and habitat use of large-bodied fishes in Boat Creek to inform the need for specific fish passage solutions; and

4) Assess status of the radio-tagged Murray cod population and radio-telemetry infrastructure and purchase equipment for upgrade of three remote logging towers.

Murray cod reproductive variance, movement, effective population size and age structure

Samples of tissue from larval, juvenile, sub-adult and adult Murray cod collected from Chowilla from 2017–2022 (*n* = 291) underwent successful DNA sequencing using the Dartseq platform. Following processing and filtering of single-nucleotide polymorphism (SNP) data, sibling relationships were identified. Half- and full-sibling relationships where then used to quantify contribution by different parents to offspring and estimate effective population size across years within Chowilla. Kinship relationships among samples from Chowilla and the nearby Lindsay-Mullaroo were used to infer movement among these systems.

Within Chowilla, Murray cod exhibited several mating systems, including within-season polygamy, between-season polygamy and monogamy, and reproductive skew (disproportionate contribution of adults). The offspring sequenced and associated with spawning years from 2017–2021 were estimated to be derived from 268 different parents. A high proportion of offspring were derived from unique parents (i.e., no full or half siblings were detected), suggesting that the breeding adult population within Chowilla is substantially larger than the 268 adults identified. Estimated values of effective population size and the ratio of number of breeding parents identified to the number offspring sequenced, varied annually. This suggests that some breeding seasons have more breeding adults contributing to reproductive output and therefore offspring collectively have higher genetic diversity. Estimates of effective population size, however, are sensitive to cohort sample size (i.e., spawn year) and the proportion of larvae in the cohort sample, and when accounted for, there was less variability among cohorts. The greatest effective population size and genetic diversity were observed from the 2017 cohort. Kinship relationships between samples from Chowilla and Lindsay-Mullaroo provide evidence of movement and demographic connectivity among the two systems. A limited sampling period and low sample numbers means the results are preliminary and interpretive power will increase with collection and sequencing of further samples. This genetic approach shows promise for providing greater insight on the potential effects of flow, structure operation and connectivity on the population dynamics of Murray cod in the region.

Murray cod epigenetic age structure

A non-lethal method for ageing Murray cod from tissue samples was trialed using a recently developed epigenetic clock approach. The clock was based on 26 evolutionarily conserved age-associated CpG methylation sites from zebrafish (*Danio rerio*) and applied to extracted DNA from 72 Murray cod sampled from Chowilla in 2021 and 2022. We were unable to obtain six of the 26 CpG sites required to fully implement the epigenetic clock of Mayne et al. (2021). As estimates were based on this reduced set of clock sites, our predicted ages for Murray cod were unreliable. Issues with limited CpG sites were confounded further by a lack of older (> 1 year) Murray cod individuals in our dataset, making rigorous assessment of age estimation difficult. To address these issues, the dataset was expanded to include sequencing read data for additional samples of Murray cod, as well as Mary River cod (*Maccullochella mariensis*) and golden perch (*Macquaria ambigua*) of known age, and CpG sites additional to those suggested by Mayne et al. (2021). Using this expanded dataset, we were able to develop a new epigenetic clock, which resulted in improved yet still unreliable age prediction (r^2cv = 0.41). In summary, the findings suggest that the non-lethal epigenetic ageing method shows promise but requires further investigation with a larger number of samples before being applied with confidence to assess age structure of Murray cod at Chowilla.

Fish movement and habitat use in Boat Creek

From June 2021–July 2022, the movements of 38 large-bodied fish (Murray cod = 7, golden perch (*Macquaria ambigua*) = 17, freshwater catfish (*Tandanus tandanus*) = 4, silver perch (*Bidyanus bidyanus*) = 1, and common carp (*Cyprinus carpio*) = 9) in Boat Creek and the broader Chowilla region were monitored using acoustic telemetry. Due to high water levels throughout the study, not all acoustic receivers could be retrieved. Nonetheless, the data obtained provides insights on differences in movement patterns among species, differing use of Boat Creek, passage through the Boat Creek bridge and use of the floodplain lakes of Chowilla.

For species with multiple tagged individuals, golden perch exhibited the greatest estimated linear extent (range = 0–219 km, mean = 53 km, *n* = 17), followed by common carp (range = 1–200 km, mean = 36 km, *n* = 9), Murray cod (range = 1–14 km, mean = 3.8 km, *n* = 9) and freshwater catfish (range = 0.3–0.8 km, mean = 0.5 km, *n* = 3). All species, with the exception of freshwater catfish, exhibited small-scale (<10 km) movements among Boat Creek and other creeks of Chowilla or the adjacent River Murray. Freshwater catfish movement was restricted to Boat Creek. Silver

perch, golden perch and common carp also exhibited instances of long-distance upstream migration, including transition to the Darling and mid-Murray rivers.

Passage through the rock bank immediately upstream of the Boat Creek bridge was demonstrated for golden perch, silver perch, Murray cod and common carp. Upstream passage occurred when water levels upstream of the Chowilla Regulator were greater than ~17 m AHD, during a period of regulator operation and during non-operation. As such, the passage of large-bodied fishes (>400 mm in length) past the Boat Creek bridge is unlikely to be impaired when QSA is ≥17,000 ML.day^{-1} and during most regulator operations that peak at ≥17 m AHD during discharges <17,000 ML.day^{-1}.

Maintenance of radio-telemetry infrastructure and summary of tagged Murray cod

The spatial ecology of Murray cod within the Chowilla system and the adjacent River Murray has been a focus of research since 2007. These studies have predominantly used radiotelemetry and been supported by a series of nine telemetered logging stations (ATS radio receiver/loggers) that have remotely monitored the movement of >100 individual Murray cod. To ensure system functionality and enable continued investigations of Murray cod movement and habitat use in the region, there is a need for periodic maintenance of radio-telemetry infrastructure and updated status of the radio-tagged Murray cod population. In 2021/22, extensive upgrades, including solar panel, solar regulator, and battery replacement, were undertaken at three loggers. As of winter 2022, a total of 46 tagged Murray cod were detected within Chowilla or the adjacent River Murray via manual tracking or on remote logging stations. Based on estimated battery life, by end-2022, the tagged Murray cod population has likely been reduced to approximately 30 fish. Of these, most tags are due to expire in 2026 and beyond. As such, the continued investigation of spatial ecology of Murray cod at Chowilla and the adjacent River Murray will be reliant on continued monitoring of existing tagged fish, further implantation of individuals with radio tags and/or transition to acoustic telemetry technology (including tag implantation) from 2023–2026.

Keywords: Murray cod, genetics, kinship, acoustic telemetry, radio telemetry.

1. INTRODUCTION

1.1. Background

The Chowilla Anabranch and Floodplain system (hereafter Chowilla) comprises the largest area of undeveloped floodplain habitat in the lower River Murray, encompassing a series of anabranching creeks, backwaters, wetlands and terminal lakes that bypass Lock and Weir No. 6 (hereafter Lock 6) on the River Murray. Due to the ~3 m head differential created by Lock 6, Chowilla exhibits permanent lotic (flowing water) habitats in what previously would have been a combination of perennial and ephemeral streams. Lotic habitats are now uncommon in the lower River Murray, where the construction of locks and weirs has transformed the river into a series of cascading weir pools that, under low flows (i.e., <10,000 $ML.day^{-1}$), are predominantly lentic (still water) in character (Bice et al. 2017, Mallen-Cooper and Zampatti 2018). The flowing creeks of Chowilla provide unique physical and hydraulic habitats that maintain remnant populations of endangered riverine fauna (e.g., Murray cod, *Maccullochella peelii*) that have declined in the main-channel weir pool habitats of the lower River Murray (Zampatti et al. 2014). The associated floodplains of the system also support significant plant communities that include river red gum (*Eucalyptus camaldulensis*) and black box (*Eucalyptus largiflorens*) woodlands, lignum (*Duma florulenta*) shrublands, chenopod shrublands, grasslands and herblands (Nicol et al. 2021).

At Chowilla, the ecological communities of both aquatic and floodplain environments have been considered degraded for some time due to the impacts of river regulation. In-channel habitats have been fragmented by barriers to flow and fish passage, while decreased flooding frequency and salinization, impact the condition and recruitment of long-lived floodplain eucalypts. Nonetheless, the site is now the focus of substantial ecological restoration efforts. Several in-channel barriers to flow and fish movement have been remediated through regulator upgrades and fishway construction (e.g., Slaney and Pipeclay weirs), whilst others remain (e.g., the Boat Creek road bridge/rock bank). Efforts to rehabilitate floodplain woodlands commenced in 2014, with the construction of the Chowilla Creek Regulator and ancillary structures, which enable large-scale engineered floodplain inundation with a primary aim of improving ‘ecological condition’ of floodplain overstory vegetation.

Operation of the Chowilla Regulator, whilst potentially promoting localised ecological benefits for floodplain biota, also poses potential ecological risks, particularly for aquatic biota that depend on lotic habitats, notably, Murray cod. These include: 1) fragmentation of habitats and obstruction of

movement; and 2) alteration of stream hydraulics (reduced water velocities). These mechanisms may act in unison to impact spawning related movements and habitat quality, and ultimately the recruitment and abundance of Murray cod (Mallen-Cooper et al. 2011, Koehn and Nicol 2014, Fredberg and Zampatti 2018). To better understand the response of Murray cod and other fish species to regulator operation and a range of other management interventions at Chowilla, an *intervention monitoring* program supports targeted investigations to inform adaptive management. This report presents the fish intervention monitoring program for 2021/22, which included three specific but related components. The background for each is provided below.

Using genetics to assess Murray cod reproductive variance, movement, effective population size, and age structure

Understanding species population dynamics is critical for management and conservation. This includes knowledge of trends in relative abundance, population size and demography (e.g., age structure), as well as other reproductive characteristics, including the relative contribution of different breeding adults. Specifically, for long-lived species, such insights may provide clarity on the impact of specific threats (e.g., regulator operation, altered hydrodynamics and low dissolved oxygen) on reproduction and recruitment that may not be immediately obvious from survey data and analysis of trends in abundance.

Genetic approaches that assess kinship relationships among cohorts of offspring (larvae and juveniles within and across spawning seasons) can be used to investigate the mating systems of species and populations. This includes providing evidence of pair bonding and polygamy/monogamy, as well as estimation of the number of successful breeding adults and reproductive variance (disproportionate contribution from individual breeders) in a population across time. An initial exploration of these parameters using tissue from larval, juvenile, sub-adult and adult Murray cod collected from Chowilla from 2017–2021 provided evidence of several mating systems, including within-season polygamy, between-season polygamy and monogamy, and reproductive skew (disproportionate contribution of adults) (Bice et al. 2023). Estimated values of effective population size and the ratio of number of breeding parents identified to the number of offspring sequenced, varied annually, suggesting that some breeding seasons have more breeding adults contributing to reproductive output and therefore offspring collectively have higher genetic diversity. Furthermore, kinship relationships between samples from Chowilla and the nearby Lindsay-Mullaroo system provided evidence of movement and demographic

connectivity between these two systems. As such, these techniques appear a viable approach to complement existing TLM Condition Monitoring and provide greater insight on Murray cod reproduction and population dynamics in the Chowilla region, including the influence of system management.

Additionally, further novel genetic techniques exist that may allow the non-destructive ageing of Murray cod (Mayne et al. 2021). Length is a poor proxy for age for large Murray cod (e.g. >500 mm in length) and as such, accurate estimation of Murray cod population demography typically relies on otolith microstructure analysis. Given the threatened status of Murray cod (listed as *vulnerable* under the EPBC Act 1999) this destructive approach is not preferred. As such, new genetic approaches provide great promise in understanding demography and have been further trialed using Murray cod tissue samples collected from Chowilla in 2021 and 2022.

Fish movement and habitat use in Boat Creek

Boat Creek is one of several influent creeks of the Chowilla system, flowing from the River Murray upstream of Lock 6 downstream to Chowilla Creek. It is divided into two distinct reaches (i.e., lower and upper) by a bridge and adjacent breached rock bank (hereafter Boat Creek Bridge). Boat Creek Bridge creates a channel constriction that may present a barrier to fish movement during regulated within channel flows (the structure is 'overtopped' during flood and regulator operations of a sufficient height). Lower Boat Creek is narrow (typically <10 m wide) yet is characterised by abundant hydraulic habitat (moderate–high water velocities) favoured by native large-bodied fishes. This reach historically supported juvenile Murray cod and moderate abundances of the *protected* (Fisheries Management Act 2007) freshwater catfish (*Tandanus tandanus*) (SARDI unpublished data). During the Millennium Drought (2001–2010), however, low flows in the lower River Murray prompted the placement of rocks (2009) at the upstream entrance of Boat Creek to reduce inflows. This has altered hydrodynamics and resulted in siltation at the junction of Boat and Chowilla creeks. Siltation has limited vessel access to the creek, and subsequently, fish assemblages in lower Boat Creek have not been sampled for over 10 years.

A fishway to facilitate fish upstream movement at the Boat Creek Bridge has been considered and a fishway scoping and design report developed (Mallen-Cooper 2009). Nonetheless, aspects of the use of lower Boat Creek by large-bodied fishes remained unknown. This includes: the abundance of various species; nature of habitat use (resident, seasonal or vagrant); and the influence of the bridge on fish movement. Acoustic telemetry provides a means of investigating

the movement of large-bodied fish within Boat Creek, which could inform management of creek hydrology (e.g. rock removal) and the potential need for a fishway. As part of intervention monitoring in 2020/21, an array of acoustic receivers was deployed in Boat Creek and broader Chowilla, and a total of 38 large-bodied fish, comprising Murray cod, golden perch (*Macquaria ambigua*), freshwater catfish (*Tandanus tandanus*), silver perch (*Bidyanus bidyanus*) and common carp (*Cyprinus carpio*) were captured from Boat Creek and implanted with acoustic transmitters. The movements of these individuals were subsequently monitored throughout 2021/22.

Maintenance of radio-telemetry infrastructure and summary of tagged Murray cod

The movement of Murray cod in Chowilla system and the adjacent River Murray has been a focus of research since 2007 (Leigh and Zampatti 2009). Studies have primarily used radiotelemetry, which have collectively involved the tagging of >100 individual Murray cod and tracking of movement both manually and remotely using nine telemetered logging stations (ATS radio receiver/loggers) located on major tributaries of Chowilla Creek, at the junction of Chowilla Creek and the River Murray, and on the Chowilla Regulator. This work has been critical in understanding the spatial ecology of Murray cod in Chowilla including identifying key habitats within the system, temporal and flow-related patterns of movement between the anabranch and the River Murray, and the influence of the Chowilla Regulator on these movements (Leigh and Zampatti 2013, Zampatti et al. 2016, Fredberg et al. 2019).

The remote logging stations consist of multiple components (radio receiver, solar panels, batteries, modems, and antennas) and require periodic maintenance and upgrades, as well as resources for database hosting. To ensure system functionality and enable continued investigations of Murray cod movement using radio-telemetry, several new components and software upgrades need to be applied. This will likely occur over time in a staggered approach, with five stations upgraded in 2021/22. In addition to infrastructure upgrades, studies of movement are reliant on maintaining a population of tagged fish. In 2021/22, the status of tagged Murray cod at Chowilla was determined. This included fish with active tags that were manually and remotely located, and those potentially present based on estimated tag battery life. The battery life of all tags will be forecast to provide estimated dates for cessation of transmission. Commentary is provided on conducting future studies with radio-telemetry and also transitioning to acoustic telemetry technology.

1.2. Objectives

In 2021/22, the primary objectives of this project were to:

1) Capture Murray cod larvae and young-of-year (YOY) derived from spawning in 2021 and, together with historic samples, undertake genetic analyses using kinship approaches to provide an estimate of reproductive variance, movement and breeding population size for comparison with previous and future spawning seasons;
2) Collect tissue samples from Murray cod captured during annual condition monitoring and undertake epigenetic analyses to estimate population age structure;
3) Monitor the movement and habitat use of large-bodied fishes in Boat Creek to inform the need for specific fish passage; and
4) Assess status of the radio-tagged Murray cod population and radio-telemetry infrastructure and purchase equipment for upgrade of three remote logging towers.

2. METHOD

2.1. Study Site

Chowilla comprises a series of anabranching creeks, backwaters, wetlands, and terminal lakes that bypass Lock 6 on the River Murray, South Australia (Figure 1). Chowilla is part of the Riverland Ramsar site, a Wetland of International Importance, and is an *Icon Site* under the Murray-Darling Basin Authority's *The Living Murray Program* (MDBA 2016).

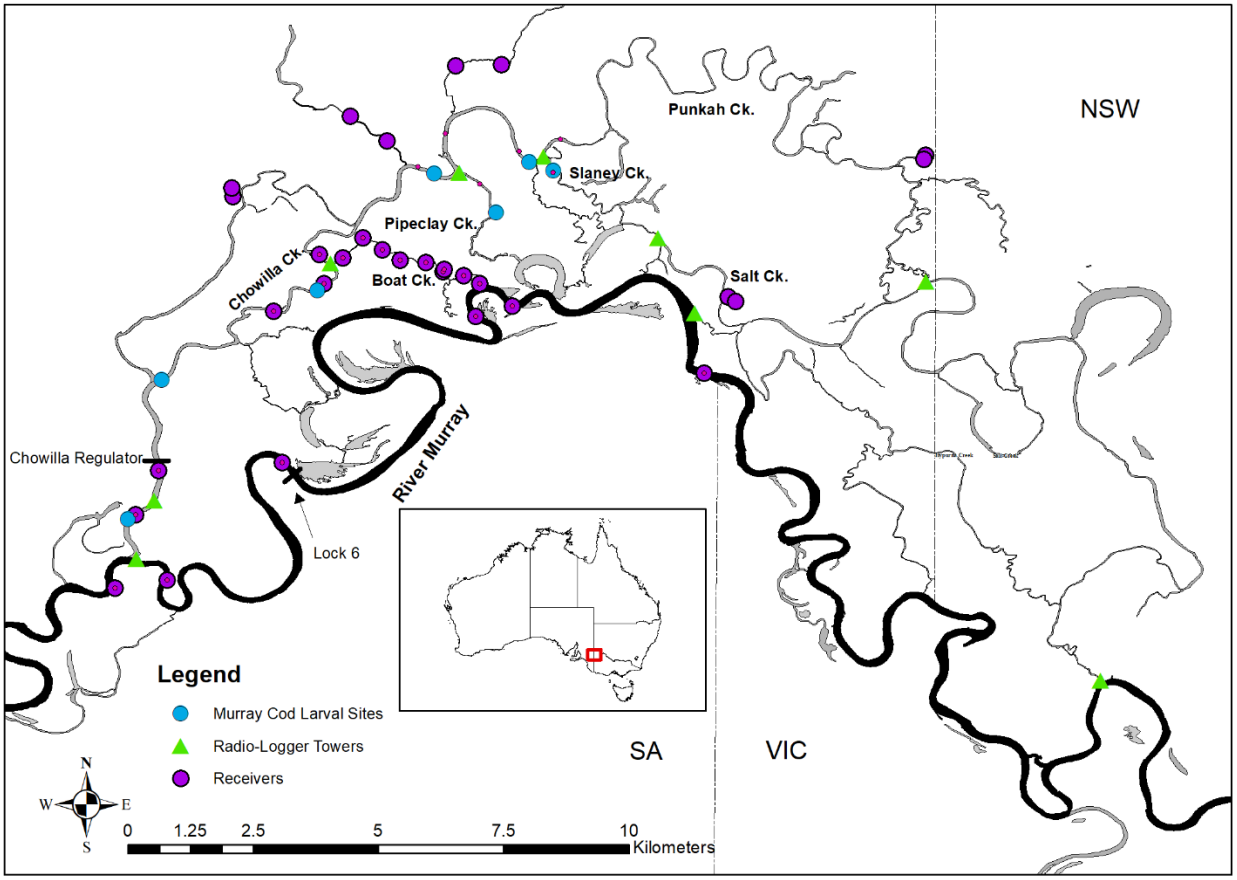

Figure 1. Map of the Chowilla Anabranch System on the River Murray, South Australia, depicting the seven sites sampled for Murray cod larvae (closed blue circles), the location of: a) 36 acoustic receivers deployed throughout the system to monitor the movement of acoustically tagged fish (closed purple circles) and b) nine fixed radio telemetry stations (logger towers) (closed green triangles).

2.2. Murray cod reproductive variance, movement, and effective population size

Sampling

Investigation of Murray cod kinship, reproductive variance, migration, and effective population size was undertaken using tissue collected as part of the current project and existing collections. From Chowilla, existing collections of larvae and YOY fish were available from 2017–2021 (see Gibbs et al. 2020, Bice et al. 2023), while in 2021/22, sampling of Murray cod derived from spawning in spring 2021 occurred via two approaches: 1) larval sampling in spring 2021; and 2) sampling of YOY Murray cod in autumn 2022 during annual condition monitoring electrofishing surveys (SARDI unpublished data). In addition, to supplement larvae and YOY sampling, tissue samples were also analysed from opportunistically collected (autumn 2019–2022) juvenile, sub-adult and adult Murray cod. Analysis of samples from these life stages potentially enables assessment of reproductive contribution from these specific individuals. Existing tissue samples of YOY, juvenile, sub-adult and adult fish captured from the Lindsay-Mullaroo system from 2018–2022 were also included to investigate movement of fish between Chowilla and Lindsay-Mullaroo.

In 2017, 2018, 2020 and 2021, larval sampling occurred during the peak spawning and drift period for Murray cod at Chowilla (October–November), at six–seven sites from Slaney Creek to lower Chowilla Creek (Figure 1; Table 1). These sites were established during a previous investigation of Murray cod spawning and larval distribution in the Chowilla system (Gibbs et al. 2020). Each site comprised an approximately 100 m long reach and was sampled overnight on multiple occasions with three drift nets (n = 1–6 nights) and six quatrefoil light traps (n = 3–6 nights) (Table 1). Drift nets were 1.5 m long and 0.5 m in diameter, tapering to a removable ‘cod end’, and constructed of 500 µm mesh with the volume of water filtered determined by means of a flowmeter (General Oceanics Inc., Florida, USA) fixed in the mouth of the net. Drift nets were set off woody debris positioned in flow to allow the sampling gear to capture larvae effectively. Quatrefoil light traps (225 x 225 x 225 mm; Floyd et al. 1984), were clad with 5 mm mesh, and lit with a yellow cyalume glow-stick (Cyalume Technologies Inc., West Springfield, MA, USA) inside the trap. Light traps were set in the littoral zone on both banks (3 x right-hand bank: 3 x left-hand bank) and were positioned adjacent to physical habitat (e.g., macrophytes and woody debris). Both net types were set at dusk and retrieved the following morning, generally by 10:00 h. Upon retrieval, the samples were preserved in situ in 95% ethanol and returned to the laboratory for processing. Larvae were removed from the samples under a dissecting microscope, and Murray cod identified and enumerated.

Table 1. Details of sites sampled for Murray cod larvae at Chowilla in 2017, 2018, 2020 and 2021, including the number of sampling events for drift nets and light traps.

	2017		2018		2020		2021	
Site name	**Drift net**	**Light trap**	**Drift net**	**Light trap**	**Drift net**	**Light trap**	**Drift net**	**Light trap**
Slaney Creek	-	*N* = 5	-	*N* = 3	*N* = 2	*N* = 3	*N* = 2	*N* = 3
Chowilla downstream Slaney	*N* = 6	*N* = 6	*N* = 5	*N* = 6	*N* = 2	*N* = 4	*N* = 2	*N* = 4
Pipeclay Creek	-	-	-	-	*N* = 2	*N* = 4	*N* = 2	*N* = 4
Chowilla downstream Pipeclay	*N* = 6	*N* = 6	*N* = 5	*N* = 6	*N* = 1	*N* = 4	*N* = 1	*N* = 4
Chowilla downstream Boat	*N* = 6	*N* = 6	*N* = 5	*N* = 6	*N* = 1	*N* = 4	*N* = 1	*N* = 4
Chowilla upstream Monoman	*N* = 6	*N* = 6	*N* = 5	*N* = 6	*N* = 1	*N* = 3	*N* = 1	*N* = 3
Chowilla downstream Regulator	*N* = 6	*N* = 6	*N* = 5	*N* = 6	*N* = 1	*N* = 3	*N* = 1	*N* = 3

In autumn of 2017–2022, tissue samples were obtained from YOY, juvenile, sub-adult and adult Murray cod collected during annual Chowilla fish condition monitoring surveys using a vessel mounted 5 kW Smith Root Model GPP electrofishing system (SARDI unpublished data). Samples from the Lindsay-Mullaroo were collected in autumn of 2018–2022 using a vessel mounted 7.5 kW Smith Root Model GPP electrofishing system (Tonkin et al. 2021). After capture, individual Murray cod were measured for length, and a fin clip taken and preserved in ethanol, before being released. For these older Murray cod, total length was used to estimate the age of each individual as YOY (0+), one year old (1+), sub-adult (2–4+) and adult (5+) using a previously published growth curve (Todd and Koehn 2009).

DNA extraction, sequencing, and SNP filtering

Murray cod tissue samples were sent to Diversity Arrays Technology (DArT: Canberra, ACT, Australia), where total genomic DNA was extracted. DNA samples were then sequenced using the Dartseq platform, which uses a form of reduced representation sequencing similar to double-digest restriction site-associated DNA sequencing. DNA samples were digested using the

restriction enzymes PstI and SphI, with two adaptors corresponding to the two different restriction enzyme overhangs, following the digestion and ligation methods described by Kilian et al. (2012). The forward (PstI) adaptor included an Illumina flow cell attachment sequence, sequencing primer sequence and a unique barcode for multiplexing, whereas the reverse adaptor included an Illumina flow cell attachment sequence. Equimolar amounts of each amplified product were then pooled and sequenced as single reads on an Illumina Hiseq 2500 for 77 cycles. Samples were sequenced in batches of 94 per Illumina sequencing lane, with 25% of samples rerun as technical replicates for quality control. Sequenced reads were processed using DArT proprietary analytical pipelines described in Kilian et al. (2012), with poor-quality sequences removed and low-quality bases corrected. A secondary pipeline (DArTsoft14) was used to compile read counts into Single-nucleotide polymorphism (SNP) loci calls. SNPs were further filtered to remove loci whose allele read counts had a greater than fivefold difference from each other, and that scored <95% reproducibility using the sequenced technical replicates.

Additional individual and SNP filters were applied to ensure a high-quality SNP dataset for analysis using the dartR v 2.1.4 package in R v 4.2.0 (Gruber et al. 2018, Mijangos et al. 2022, Team 2023). We retained only those SNPs that were consistent in at least 98% of technical replicates and that were present in at least 70% of individuals. Individuals missing data for more than 30% of loci were removed. We also filtered SNPs to remove monomorphic loci and loci where minor alleles were present in <2 % of all individuals and retained only a single SNP per sequence tag.

Kinship analysis and assignments using Colony

Kin relationships (full-sibling, half-sibling, and parent-offspring pairs) were identified using Colony2 v2.0.6.8 (Jones and Wang 2010, Wang 2012). Colony was run three times for the entire dataset (treating non-adult life stage as offspring and adult individuals as candidate parents). The analysis was run with the following parameter settings: Error rate for each locus 0.001; Polygamy for males and females (i.e., to allow half-siblings); Non-inbreeding (recommended for dioecious with no or low level of inbreeding); Dioecious diploid; Sibship size scaling; No sibship prior; Calculating allele frequencies; Not updating allele frequencies; Medium run length; Full-likelihood method and high-precision. Markers were assumed to be codominant. Only those kin pairs that were detected in all three replicate runs with a probability of > 99% were retained.

Identifying repeat breeding events among adults and the estimated number of breeding adults per year

Using the BestCluster output file from the Colony analysis, we calculated the number of breeding adults contributing to sampled offspring across the entire dataset and for each cohort separately. We also calculated the number of breeding adults who contributed to multiple offspring cohorts, and the number of adults engaging in polygamy within- and between- breeding seasons. For the whole dataset we calculated the cumulative contribution of each parent across all breeding seasons to measure the reproductive variance among adults.

Identifying movement events between Chowilla and Mullaroo for sequenced individuals

The Colony output files containing all predicted full-sibling and half-sibling pairs at >99% confidence were interrogated and the number of sibling pairs found in the same location and from different locations was calculated. This information was used to quantify movement events between the two systems.

Calculating effective population sizes in Chowilla

The effective population size (Ne) of the Chowilla Murray cod population was calculated using two methods: 1) the single sample linkage disequilibrium (LD) method implemented in the program NeEstimator (Do et al. 2014); and 2) the sibship-based estimation method implemented in Colony2 (Wang 2009). Effective population size estimates were obtained using both methods for each Chowilla cohort (spawn year) separately and for the total adult sample across Chowilla and Lindsay-Mullaroo. The LD method was implemented assuming random mating with a threshold frequency of 0.01 for removing rare alleles and calculating 95% confidence intervals using the jack-knife-across-samples method. Colony was implemented using the same Colony run parameters as described above (see *Kinship analysis*).

For a single cohort of an iteroparous species, the effective population size estimates should reflect the effective number of breeders in one breeding cycle (considered to reflect short-term effective population size and inbreeding in the parental generation). For a mixed age sample of adults with unknown ages, the LD method is more appropriate than the Colony method, and estimates will reflect effective size per generation (Ne; important for long-term evolutionary processes). LD method estimates were adjusted to correct for age structure bias using two life-history traits (adult lifespan (AL) = 30 and age at maturity (alpha) = 5), following the method outlined of Waples et al.

(2014). Given the samples were drawn from a number of cohorts approximately equal to a generation length (in our case ~5), age structure bias should not be too strong and our sampling should give reasonable estimates of *N*e per generation (Waples et al. 2014).

2.3. Epigenetic ageing

For investigation of Murray cod population age structure, the epigenetic clock developed by Mayne et al. (2021) was applied. The clock was based 26 evolutionarily conserved age-associated CpG methylation sites from zebrafish (*Danio rerio*).

Laboratory methods

DNA was extracted from 72 Murray cod samples of varying length (including 11 individuals with otolith age 0 and 10 individuals with otolith age 1) using the Qiagen DNeasyR Blood & Tissue Kit. We applied bisulphite conversion of these 72 Murray cod samples plus 20 previously extracted golden perch (of known otolith age 1–24) using the EZ DNA Methylation-Gold Kit (Zymo Research).

Some initial troubleshooting and optimization of the PCR protocols was required. Due to high levels of DNA degradation, only 47 of the 72 Murray cod samples were able to be included in the final sequencing preparation (the Bisulphite treatment degrades DNA which makes it difficult to apply to already degraded samples). The final reaction volumes for PCR were: 5 ul Qiagen Multiplex PCR Master Mix, 2 ul primer pool (the primer pool was made up at 250nM per primer), 3 ul Bisulfite treated DNA (total reaction volume 10 ul) and the final conditions for PCR were: an initial denaturation step of 95°C for 15 min, followed by 30 cycles of 94°C for 30s, 59°C for 90s and 72°C for 90s, and a final extension time of 72°C for 10 min. A second PCR was then undertaken to add Illumina sequencing adaptors and sample indexes, using: 7.25 ul Qiagen Multiplex PCR Master Mix, 4 ul water, 0.75 ul Index primer pair (10 μM), 3 ul of the initial PCR product (total reaction volume 15 ul) and an initial denaturation step of 95°C for 15 min, followed by 10 cycles of 94°C for 30s, 60°C for 90s and 72°C for 90s, and a final extension time of 72°C for 10 min. PCR products were cleaned using a magnetic bead clean (Ampure XP protocol using SPRI magnetic bead mix) and final products were normalised and pooled for sequencing using MiSeq Reagent Kit v2 Micro (300 cycles, 2x 150 bp paired end reads).

Data analysis and age estimation

Paired end reads were merged using PEAR (Zhang et al. 2014) and aligned using Bismark (Krueger and Andrew 2011) to a reduced subset of the Murray cod genome (GCA_002120245.1 mcod version 1) that consisted of only those scaffolds that were reported as containing the 26 epigenetic clock sites. The alignment parameters used were --bowtie2-N1-L 15 -bam -p 2 -score L, -0.6, -0.6 –non_directional. Methylation calling was undertaken using the *bismark_methylation_extractor* function using default parameters. To apply the clock, coverage files generated in Bismark were imported into R using the package bsseq (Hansen et al. 2012) and methylation data were converted to percentages rather than proportions. Age for Murray cod individuals was then predicted using the multivariate regression model function (*multivariatePredictorCoef*) from Horvath et al. (2022) using the clock coefficients from Mayne et al. (2021). Because age data were natural log-transformed in the Mayne et al. (2021) prior to developing the clock, we inverse log linear transformed predicted age values to give age in years. Because we were unable to obtain methylation data for 6 of the 26 epigenetic clock sites reported in Mayne et al. (2021) (see results), our age estimates were based on a reduced set of clock sites and therefore unreliable. To try and address this we attempted to increase the number of clock sites by using all CpG sites in the data (not just the published clock sites from Mayne et al. 2021). To do this we combined our sequencing read data for Murray cod and golden perch with that of Mayne et al. (2021); which included read data for Murray cod and Mary River cod individuals of known age) and aligned the reads to the entire Murray cod genome (GCA_002120245.1 mcod version 1) rather the reduced subset of scaffolds. Sequencing reads from Mayne et al. (2021) were trimmed by 15 bp at both ends and combined with our merged reads. Default alignment parameters were used (-score L, 0, -0.2). As above, methylation calling was undertaken using the *bismark_methylation_extractor* function using default parameters and Bismark coverage files were imported into R using bsseq.

A total of 297 CpG sites were detected in the combined sequence data across 32 scaffolds (but with varying coverage). The methylation data were filtered to only CpG sites with <70 missing values, then only individuals with <20 missing values. One additional sample that seemed to be an outlier was removed (GP16). We developed the clock in R using code from (Robeck et al. 2021). We used 60 samples of known age (1 Mary River cod 0.5 years; 50 Murray cod 0.5–12.1 years; 9 golden perch 2–24 year). Age was log transformed and missing methylation values were imputed (based on the means of the non-missing values). To determine whether there was a

relationship between age and methylation levels, we fitted a single glmnet model with the glmnet (Friedman et al. 2010) R package (Kuhn 2008), using 10-fold cross validation to tune the model parameters. The parameters family="gaussian", alpha=0.5, nfolds = 10 were used to fit the model. We used leave-one-out cross validation to estimate the model's predictive capacity (i.e., how well the model performs for an individual whose data was not used to fit the model).

2.4. Fish movement and habitat use in Boat Creek

Acoustic receiver array and download

In June 2021, an array of 36 VEMCO VR2W acoustic receivers was deployed at Chowilla (Figure 1). The array was deployed in an arrangement that enabled: 1) fine-scale tracking of fish movement within Boat Creek (receivers approximately every 500 m); 2) assessment of passage and delay at the Boat Creek Bridge; 3) assessment of broader-scale movements among major creeks within the Chowilla system and the River Murray; and 4) assessment of use of specific floodplain lakes when inundated (i.e. Werta Wert, Coombool Swamp, Lake Limbra, Lake Littra and Punkah Horeshoe). In addition to project specific receivers, a further broader array of acoustic receivers exists across the lower Murray, mid Murray, and lower Darling rivers, which are maintained by SARDI, NSW DPI Fisheries and Victorian DELWP. Detection data from fish tagged in Boat Creek from these receivers were also considered to provide a broader spatial and temporal perspective on movement. All receivers were deployed using stainless steel cable and attached to snags or manmade structures (e.g., water quality stations, buoylines at flow regulating structures). From 9–15 June 2022, acoustic receivers were downloaded in the study reach and more broadly across the lower River Murray.

Fish Capture and Surgery

From 5–9 July and 13–16 December 2021, 38 large-bodied fish were captured from Boat Creek and implanted with acoustic tags (Table 2). Tagged fish comprised four native species, namely Murray cod (*n* = 7; 360–470 mm TL; 639–1425 g), golden perch (*n* = 17; 364–494 mm TL; 745–2000 g), freshwater catfish (*n* = 4; 318–580 mm TL; 257–1500 g) and silver perch (*n* = 1; 382 mm FL; 782 g), and non-native common carp (*n* = 9; 318–685 mm FL; 592–4692 g) (Table 2). Most individuals (*n* = 24) were tagged downstream of the bridge, with fewer tagged upstream (*n* = 14). All fish were captured using a vessel mounted 5 kW Smith Root Model GPP electrofishing system.

Following capture, fish were anaesthetised using a 0.05 ml. L^{-1} solution of AQUI-S in a 20 L dosing tank. When fully anaesthetised – characterised by loss of equilibrium and unresponsiveness to stimulus – fish were weighed (g) and measured (mm fork or total length – FL/TL) and placed ventral side up into a V-shaped support. During surgery, a 0.02 ml. L^{-1} solution of AQUI-S solution was irrigated over the gills to maintain anesthesia. For native species, a small incision was made off-centre on the ventral surface, midway between the pelvic and anal fins, through which both acoustic and PIT tags were inserted into the peritoneal cavity. For common carp, the incision was made on the fish's flank approximately 20% of the body depth above the ventral surface. All fish were fitted with a VEMCO model V9-2x (dimensions 29 x 9 mm; weight 4.7 g in air) acoustic tag. These tags have a random delay of 70–130 seconds, and estimated battery life of 747 days (estimated expiry July 2023). Combined tag weight (V9 plus the PIT tag) aimed to maintain a transmitter to fish weight ratio of <2% (Jepsen et al. 2002). Incisions were closed using a single cruciate suture. Following full recovery (i.e. fish able to maintain their balance and freely swim) in an aerated tank, fish were released at or near their original capture location.

Table 2. Capture location, acoustic ID and biological details of 38 fish implanted with V9-2x acoustic tags in Boat Creek in 2021.

Species	Date Tagged	Tagging Location	Length (mm)	Weight (g)	Acoustic Tag ID	PIT Tag ID
Native Species						
Golden perch	8/07/2021	Boat Ck US bridge	403	1078	60347-1390470	982000405336317
Golden perch	8/07/2021	Boat Ck US bridge	408	982	60359-1390482	982000405336302
Golden perch	8/07/2021	Boat Ck US bridge	456	1745	60346-1390469	982000405336329
Golden perch	14/12/2021	Boat Ck DS bridge	480	1700	60344-1390467	982000405336242
Golden perch	14/12/2021	Boat Ck DS bridge	364	842	60345-1390468	982000405336298
Golden perch	14/12/2021	Boat Ck DS bridge	473	1800	60334-1390457	982000405336322
Golden perch	15/12/2021	Boat Ck US bridge	494	2000	63320-1390871	982000405336324
Golden perch	15/12/2021	Boat Ck US bridge	485	1739	63322-1390873	982000405336333
Golden perch	15/12/2021	Boat Ck DS bridge	390	1065	60336-1390459	982000405336281
Golden perch	15/12/2021	Boat Ck DS bridge	392	891	60337-1390460	982000405336282
Golden perch	15/12/2021	Boat Ck DS bridge	400	984	60338-1390461	982000405336267
Golden perch	15/12/2021	Boat Ck DS bridge	396	970	60335-1390458	982000405336245
Golden perch	16/12/2021	Boat Ck DS bridge	435	1466	60329-1390452	982000405336292
Golden perch	16/12/2021	Boat Ck DS bridge	421	1089	60325-1390448	982000405336257
Golden perch	16/12/2021	Boat Ck DS bridge	410	1298	60328-1390451	982000405336275
Golden perch	16/12/2021	Boat Ck US bridge	385	745	60331-1390454	982000405336304
Golden perch	16/12/2021	Boat Ck US bridge	389	992	60330-1390453	982000405336310
Murray cod	6/07/2021	Boat Ck DS bridge	398	985	60354-1390477	982000405336260
Murray cod	7/07/2021	Boat Ck DS bridge	470	1425	60356-1390479	982000405336273
Murray cod	7/07/2021	Boat Ck US bridge	418	998	60358-1390481	982000405336318
Murray cod	8/07/2021	Boat Ck DS bridge	395	923	60348-1390471	982000405336279
Murray cod	14/12/2021	Boat Ck DS bridge	415	1000	60332-1390455	982000405336258
Murray cod	15/12/2021	Boat Ck DS bridge	456	1453	63321-1390872	982000405336253
Murray cod	15/12/2021	Boat Ck US bridge	360	639	60326-1390449	982000405336305
Silver perch	6/07/2021	Boat Ck DS bridge	382	782	60355-1390478	982000405336334
Freshwater catfish	6/07/2021	Boat Ck US bridge	318	257	60353-1390476	982000405336268
Freshwater catfish	7/07/2021	Boat Ck US bridge	470	1173	60357-1390480	982000405336256
Freshwater catfish	15/12/2021	Boat Ck DS bridge	395	504	60327-1390450	982000405336295
Freshwater catfish	15/12/2021	Boat Ck US bridge	580	1500	63323-1390874	982000405336297

Table 2 continued.

Species	Date Tagged	Tagging Location	Length (mm)	Weight (g)	Acoustic Tag ID	PIT Tag ID
Non-native Species						
Common carp	8/07/2021	Boat Ck DS bridge	442	1792	60349-1390472	982000405336316
Common carp	8/07/2021	Boat Ck DS bridge	408	1420	60350-1390473	982000405336265
Common carp	8/07/2021	Boat Ck US bridge	685	4629	60351-1390474	982000405336319
Common carp	8/07/2021	Boat Ck DS bridge	345	790	60352-1390475	982000405336315
Common carp	8/07/2021	Boat Ck US bridge	446	1600	60339-1390462	982000405336296
Common carp	9/07/2021	Boat Ck DS bridge	467	1799	60343-1390466	982000405336244
Common carp	9/07/2021	Boat Ck DS bridge	586	3428	60342-1390465	982000405336239
Common carp	9/07/2021	Boat Ck DS bridge	450	1650	60341-1390464	982000405336303
Common carp	9/07/2021	Boat Ck DS bridge	318	592	60340-1390463	982000405336290

Analysis of movement data

Data on tagging locations, acoustic detections and PIT reader detections were combined to create an overall movement data set. Each tagging location, acoustic receiver and PIT reader within Chowilla and the River Murray was assigned a relative river distance (± km) from a reference receiver in lower Boat Creek (i.e., BCX10, ~750 m upstream of the Chowilla Creek–Boat Creek junction). These distances were used in subsequent analyses to assess displacement from Boat Creek.

To assess meso-scale habitat use within Chowilla, the number of individual fish detected on each receiver was determined and plotted. To assess broader-scale longitudinal movements, the movement of each individual fish was plotted over the course of the monitoring period (July 2021–June 2022) with reference to displacement from Boat Creek. These data were also used to estimate linear extent, defined as the distance (km) between the most downstream and upstream detections for each fish. Ultimate destination (i.e., last detection) was also summarised for every individual.

Movement of individuals past the Boat Creek Bridge was investigated by interrogating detection data from receivers immediately downstream and upstream of the bridge. Sequential detection on these receivers indicated a passage event and the specific date and time of passage was estimated as the mid-point between these detections. For every passage event, data on discharge at the South Australian border and water level immediately upstream of the Chowilla Regulator were collated to assess the conditions under which upstream and downstream passage occurred.

2.5. Maintenance of telemetry infrastructure and tag status of tagged Murray cod

Infrastructure maintenance

There are nine remote logging stations (i.e. receivers and towers) at major creek junctions within Chowilla (Figure 1). Key internal components include: an ATS radio receiver (model R4520C); ATS RDP 800 (remote data platform); a KLK 300 industrial computer; a Maxon model MA 20-25 modem; and one or two AGM (amalgam glass mat) batteries (95 A/h). Key external components include: three, directional yagi radio antenna; single 3G antenna; and solar panel(s). As a fish passes within ~600 m of a logger tower its unique code is received by the ATS receiver via one of the three directional antennas. This data is stored on the ATS receiver and every hour a copy

is stored on the ATS Remote Data Platform. Once a day the KLK 3000 gathers a copy of the data and transmits it via email over the Telstra network to Karltek which hosts an online database for fish movement data and tower diagnostics.

In 2020/21, intermittent power supply problems and redundant software issues were identified by Karltek with upgrades needed for all nine logging stations. As part of the 2020/21 intervention monitoring project, software upgrades to the KLK3000 units were performed on all logger stations, whilst full upgrades, eliminating power supply problems, were performed on two logging stations at the junction of Slaney and Chowilla creeks and at the junction of Chowilla Creek and the River Murray. In 2021/22, further major upgrades were planned for stations at 1) the junction of Salt and Slaney creeks, 2) the junction of Salt and Punkah creeks, and 3) the junction of the River Murray and Swifty's Creek. Additionally, minor upgrades were also planned for remaining logging stations.

Status of tagged fish population

To determine the status of radio tagged Murray cod still active within Chowilla, a combination of remote and manual tracking was undertaken. Manual tracking occurred in May 2022, whereby core Murray cod habitat within Chowilla (Slaney Creek, Chowilla Creek, Pipeclay Creek, Bank K, Swifty's Creek, Salt Creek) and the adjacent Murray River main channel were tracked using a portable ATS R4500C receiver and hand-held Yagi antenna. The position of each tracked fish was determined as the point of greatest signal strength and this location recorded with a GPS and field tablet installed with ArcGIS Online. Nine remote loggers, located at the junctions of Chowilla Creek and major tributaries of the system (Figure 1), were used to assess movement and determine the location of fish before and after the manual tracking event.

3. RESULTS

3.1. Murray cod reproductive variance, movement, and effective population size

Sampling and sequencing

From tissue samples collected from Murray cod captured from 2017–2022, a total of 290 individuals from five size classes have been successfully genotyped (Table 3). This includes a total of 85 larvae and one YOY collected in 2021/22 and derived from spawning in 2021.

Table 3. Number and sizes of Murray cod from Chowilla successfully genotyped across sampling years.

Size Class	2017/18	2018/19	2020/21	2021/22	Total
Larvae (10–20 mm TL)	43	58	14	85	**199**
YOY (20–200 mm TL)	8	3	6	1	**18**
Juvenile (1 yr) (200–400 mm TL)	-	1	8	1	**10**
Sub-adult (2–4 yr) (400–600 mm TL)	-	2	18	11	**31**
Adult (600 mm+ TL)	3	13	5	11	**32**
Total	**54**	**77**	**50**	**109**	**290**

Kinship and movement among Chowilla and Lindsay-Mullaroo

A total of 235 full sibling pairs (34 full sibling groups) and 232 half-sibling pairs were detected at Chowilla (Table 4). Multiple reproductive patterns were elucidated from sibling relationships, including: adults that undertook repeat breeding events with different partners within the same season (within-season polygamy); adults that undertook breeding events with different partners across two or more seasons (between season polygamy); and adults that undertook repeat breeding events with the same partner across seasons (i.e., repeated mate pairing, monogamy). Most kinship assignments were between individuals sampled in the larval stage (233/235 full-sib pairs and 223/232 half-sibling pairs) (Tables 4 and 5) and individuals sampled from the same cohort (230/235 full-sibling and 179/232 half-sibling pairs) (Tables 3 and 4). A substantially higher number of full-sibling pairs (n = 141, 14 full-sibling groups; 2–4 individuals per sibling group) were

detected from the 2021 cohort compared to previous cohorts (0–62) (Table 4). Half-siblings, were relatively common among cohorts (53/232) indicating many of the same individuals bred repeatedly over the 5-year study period (Table 5). There were two instances of full siblings among cohorts (both full-sibling larvae sampled in 2017 and 2018) providing evidence of repeat pairing. Three kin pairs (2 full-sibling and 1 half-sibling pairs) were detected between Chowilla and Lindsay-Mullaroo indicating movement among the two systems.

Table 4. The number of full- and half-sibling pairs detected by Colony in Chowilla (based on analysis of the full dataset with candidate parents included). *Cohort* is based on the year of spawning not year of sampling and so includes all non-adult life stages as assigned to spawn based on length. The numbers of larvae and other non-adult life stage individuals (young-of-year, one-year old and juvenile) per cohort are given. Kin pairs are reported for within-cohort (which is excluding pairs sampled across different cohorts).

Cohort	Cohort *n*	Larvae *n*	YOY *n*	Full-sib groups	Full-sib dyads	Half-sib dyads
2017	63	43	20	7	22	18
2018	74	58	16	7	62	126
2019	11	0	11	0	0	0
2020	21	14	7	5	5	1
2021	86	85	1	14	141	34
Total	255	200	55	34	235	232

Table 5. Number of half-sibling pairs across Chowilla that were detected within and between cohort pairs.

Cohort	2017	2018	2019	2020	2021
2017	18				
2018	10	126			
2019	1	3	0		
2020	4	5	0	1	
2021	20	9	0	1	34

Adult contributions to breeding outputs within Chowilla

A total of 268 breeding adults were detected across the study period and among individual years, the number of breeding adults ranged from 22–92 (Table 6). Overall, most adults (72%) contributed to one sequenced offspring, but 6% of adults were found to contribute to five or more offspring across years, and one adult contributed to 20 offspring. The average number of offspring each adult contributed towards varied between years ranging from 1.00 (every offspring had a

unique pair of adults) for the 2019 cohort to 2.23 for the 2021 cohort. The number of adults found, increased with the number of offspring sequenced (i.e., most years with more offspring sampled also showed more inferred breeding adults), but the proportion of larvae sequenced (versus older juveniles) appeared to correspond with a decrease in the number of adults identified.

Table 6. Numbers of breeding adults inferred to have contributed to the recruitment of all sequenced offspring across years at Chowilla. *Cohort* is based on the year of spawning. Also presented are cohort sample size, % of offspring comprised of larvae compared to other life stages (e.g., YOY), ration breeding adults to inferred offspring, and mean and range in number of offspring contributed by breeding adults.

Cohort	Cohort *n*	%larvae	Breeding adults *n*	Ratio breeding adults to offspring	Mean offspring contributed by adults	Range offspring contributed by adults
2017	63	68%	82	1.30	1.42	1–5
2018	74	78%	92	1.24	1.48	1–20
2019	11	0%	22	2.00	1.0	1
2020	21	67%	27	1.29	1.41	1–2
2021	86	99%	69	0.80	2.23	1–12
All years	255	78%	268	1.05	1.72	1–20

Effective population sizes for Chowilla

Per cohort (including larvae and juveniles) effective population size estimates ranged from 49.2–113.9 and 41–128 for the LD and Colony methods, respectively (Table 7). Irrespective of method, the 2017 cohort had the largest effective population size estimate (LD = 113.9 and Colony = 128; Table 7). Because the 2021 cohort was comprised almost entirely of larvae (99%), we also estimated effective population sizes for the other cohorts using just the larval samples for comparison, as the inclusion of older juveniles has been shown to lead to higher effective population size estimates (O'Dwyer 2022). Based on larvae alone, effective population sizes were generally lower and more similar across cohorts (25-61; Table 7), yet, the 2017 cohort still had the largest effective population size (Table 7). Effective population size estimates for the total adult (5+ years) sample across

Chowilla (*n* = 32) were 679.0 (198.9–Infinity) and 661 (310–Infinity) for the LD method (corrected) and the Colony method, respectively.

Table 7. Effective population (Ne) size estimates of different cohorts (estimated spawn year) of Murray cod sampled from Chowilla. Estimates are provided using the LD and Colony methods. For each cohort, estimates are provided using just larvae (l) and a combination larvae and juveniles sampled in subsequent years that were assigned to that spawning year based on the age-length growth curve.

			Larvae & juvenile		**Larvae**	
Cohort	***N***	***N* larvae**	**Ne (LD, corrected)**	**Ne (Colony)**	**Ne (LD corrected)**	**Ne (Colony)**
2017	63	43	113.9 (69.6–252.5)	128 (92–179)	57.6 (35.5–119.5)	61 (40–101)
2018	74	58	70.3 (47.9–115.0)	41 (27–64)	46.1 (31.9–76.0)	25 (15–46)
2019	11	0	NA*	NA*	NA*	NA*
2020	21	14	71.6 (38.5–300.4)	93 (52–344)	39.1 (19.3–257.5)	52 (26–191)
2021	86	85	49.2 (36.2–69.7)	44 (29–67)	48.0 (35.3–67.8)	43 (29–65)
Total	255	200	679 (198.9–∞)	661 (310–∞)		

3.2. Murray cod epi-genetic ageing

Applying the Murray cod specific epigenetic clock using the CpG 20 sites for which data was available, three individuals returned unrealistic high age estimates (2 were > 60,000 years old and another 299 years), which may reflect an abnormal methylation signal driven by sample quality. When these three outlier individuals were removed, predicted ages were in the expected range (0-12 years) and there appeared to be a weak positive relationship between predicted age and length. Nonetheless, the foundation of the clock on 20 rather than 26 methylation sites dictates that age estimation was likely less accurate than that reported by Mayne et al. (2021).

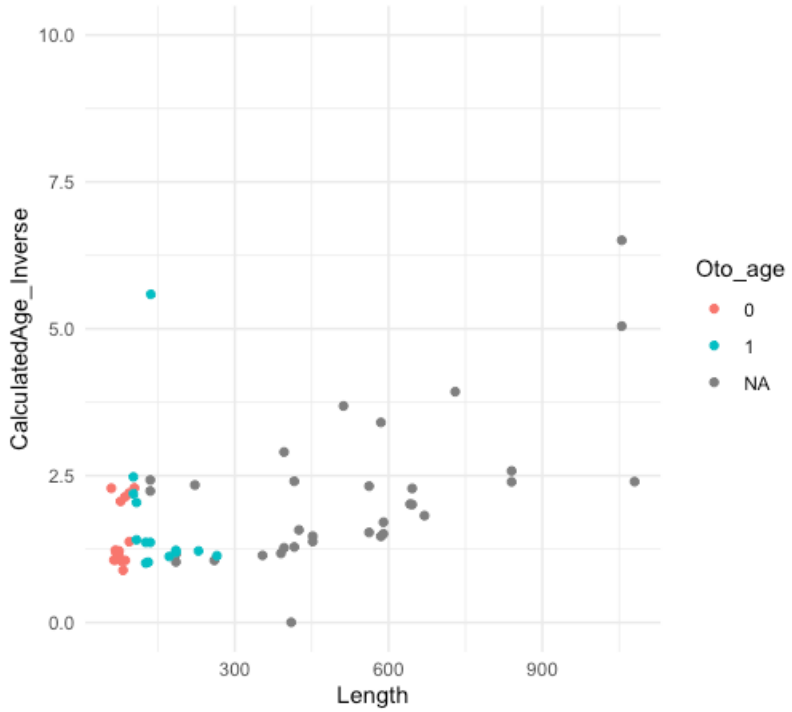

Figure 2. Predicted age (in years) of Murray cod against total length (mm) based on Murray cod specific epigenetic clock. Individuals determined to be 0+ or 1+ years of age-based otolith microstructure are indicated in red and blue, respectively.

For the clock we developed using Murray cod, Mary River cod and golden perch, and all the CpG sites contained in the sequence data (33 sites were retained post-filtering), there was a positive relationship between fitted age (based on methylation data) and otolith age (r^2 = 0.95), although this relationship had only moderate predictive capacity (r^2_{cv} = 0.41) (Figure 3). Limited predictive power of the model is not unexpected given the relatively small sample size (n = 60) and the fact that there were very few fish older than 10 in the dataset (n = 7). We fitted the same model using Murray cod and Mary River cod individuals only, but the model based on this reduced data set performed worse (r^2 = 0.58 and r^2_{cv} = 0. 14). Inclusion of golden perch increased the predictive power of the model, most likely because including golden perch substantially increased the range of ages included in the data set (all fish older than 12 years in the dataset were golden perch).

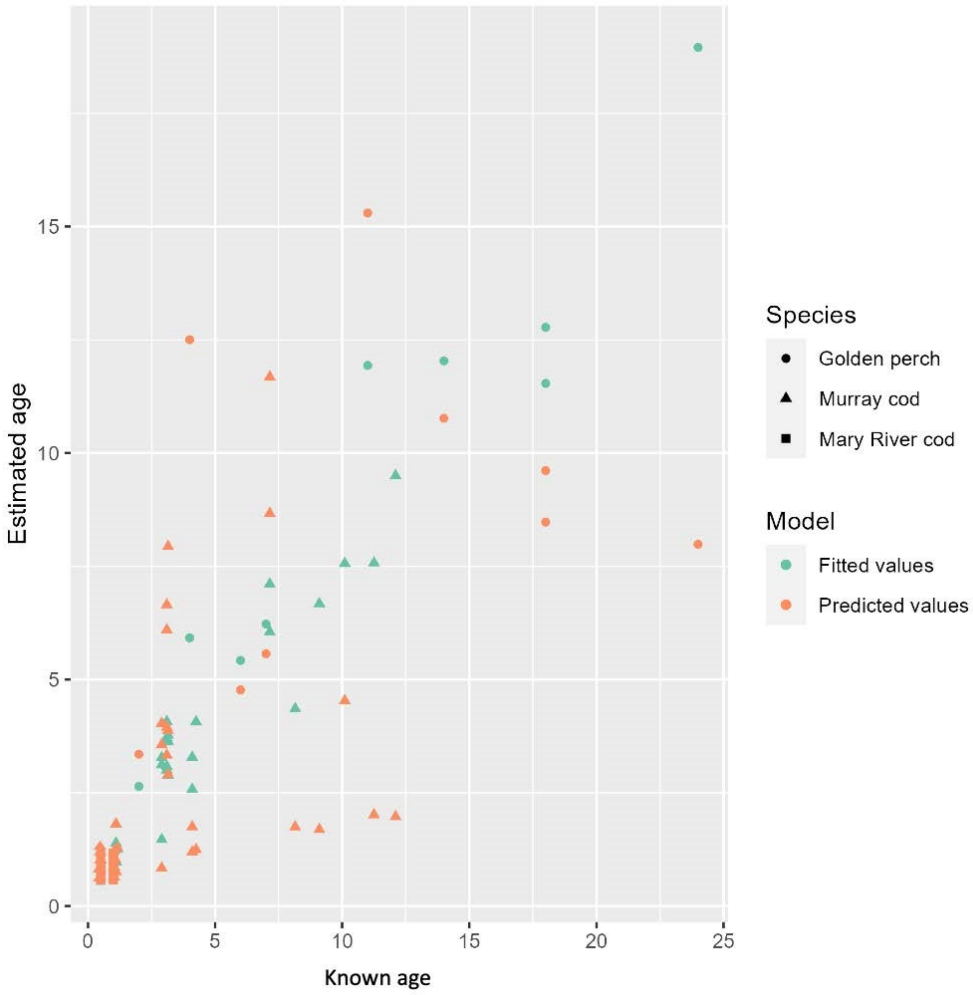

Figure 3. Correlation plot of known age (otolith or captivity) verse estimated age for Murray cod, Mary River cod and golden perch based on the epigenetic clock using data from the current project and sequence data from Mayne et al. (2021) and all CpG sites contained in the sequence data.

3.3. Fish movement and habitat use in Boat Creek

Hydrology

From June 2021–July 2022, discharge in the lower River Murray (QSA) was variable. During June 2021, when receiver deployment occurred, discharge was generally <8000 ML.day^{-1}, but increased rapidly in August to >20,000 ML.day^{-1} (Figure 4). Discharge increased further in late September and remained >25,000 ML.day^{-1} until February 2022, with peaks of ~35,000 ML.day^{-1} in mid-November and late-December 2021. Discharge receded to ~17,000 ML.day^{-1} in March

2022 before gradually increasing to >30,000 ML.day^{-1} in June 2022. Operation of the Chowilla Regulator occurred from 2 August–30 November 2021. Water level immediately upstream of the regulator rose rapidly throughout August before reaching ~19.5 m AHD and being maintained around this level for September and October (Figure 4). Recession occurred throughout November, after which the regulator was 'open' and water level subsequently varied with QSA.

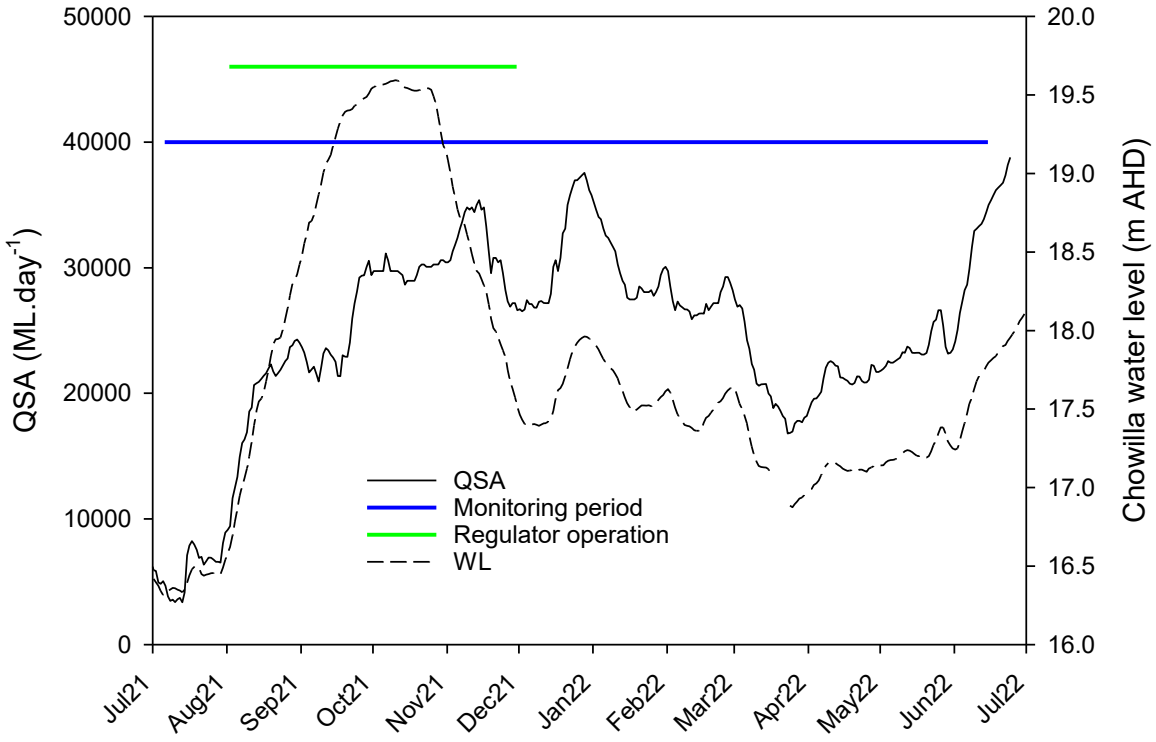

Figure 4. Discharge in the lower River Murray (solid black line, QSA, ML.day^{-1}) and water levels within Chowilla (dashed black line, m AHD) from July 2021–June 2022. Period of acoustic tracking (blue line) reported upon and managed floodplain inundations at Chowilla (green line) are also indicated.

Movement patterns

A total of 37 of 38 tagged fish (97%) were detected from June 2021–July 2022. Detection periods for individuals (i.e., the number of days between tagging and last detection) ranged 1–343 d (mean ± SD = 193 ± 20 d). Within Chowilla, the greatest numbers of individual fish were detected on receivers in lower Boat Creek (*n* = 23–24), with lower numbers in upper Boat Creek (*n* = 14–

16) generally reflecting the number of fish tagged in each of these reaches (Figure 5). 13 individuals were detected on a receiver in Chowilla Creek downstream of the Boat Creek junction, while lower numbers (3–6) were detected in lower Chowilla Creek, Slaney Creek, the River Murray main channel and creeks connecting Chowilla Creek to Coombool Swamp and Lake Limbra (i.e., Hancock Creek). Several individuals were also detected by receivers beyond Chowilla in the Murray and Darling rivers.

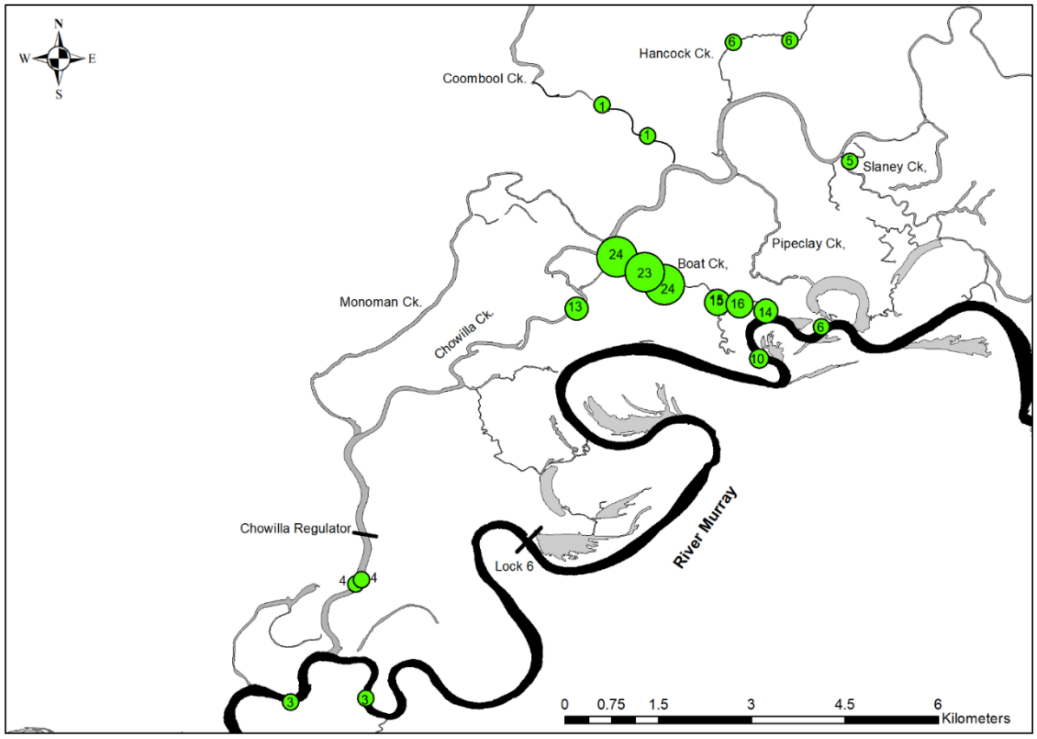

Figure 5. Number of tagged fish detected on each receiver within Chowilla and the adjacent River Murray during the monitoring period (6 July 2021 – 15 June 2022).

Patterns of movement varied among species. For species with multiple tagged individuals, golden perch exhibited the greatest estimated linear extent (range = 0–219 km, mean = 53 km, *n* = 17), followed by common carp (range = 1–200 km, mean = 36 km, *n* = 9), Murray cod (range = 1–14 km, mean = 3.8 km, *n* = 9) and freshwater catfish (range = 0.3–0.8 km, mean = 0.5 km, *n* = 3) (Figure 6–8). Only one silver perch was tagged in the study and exhibited a linear range of >250 km.

Individual golden perch exhibited variable movement. Four individuals left Chowilla and travelled long distances (~200 km) upstream in the River Murray from September 2021–March 2022 (Figure 6a). Three of these were last detected entering the lower Darling River (Figure 9a). Another individual travelled as far upstream as Lock 7 before returning to Boat Creek. The remaining individuals exhibited local movements within Boat Creek, and between Boat Creek and other creeks within the Chowilla system (e.g., Slaney Creek) and the River Murray. As of July 2022, a total of 13 fish remained within the Chowilla system or nearby (e.g., within 10 km) in the River Murray (Figure 9).

Individual common carp also exhibited variable movement. A single individual made a rapid long distance (>200 km) upstream movement in December 2021 and was last detected in the lower Darling River (Figure 7b and 9). Another individual travelled as far upstream as Lock 7 before returning to Boat Creek. The remaining fish moved extensively within Boat Creek and between Boat Creek and Chowilla Creek, and specific floodplain lakes within Chowilla. Of the nine tagged common carp, five were detected moving between Chowilla Creek and Lake Limbra, and one between Chowilla Creek and Coombool Swamp during regulator operation between 1 September and 25 October 2022. This included an individual that ultimately moved upstream to the Darling River. Occupation of floodplain lakes occurred for durations of 1–26 d and all individuals that moved into lakes subsequently returned to Chowilla Creek.

Murray cod (Figure 7b) and freshwater catfish (Figure 8) exhibited more limited movement relative to the other species and all detected individuals spent most of the study within Boat Creek. Two Murray cod exhibited small scale movements between Boat Creek and the River Murray, while three individuals exhibited movements between Boat Creek and Chowilla Creek.

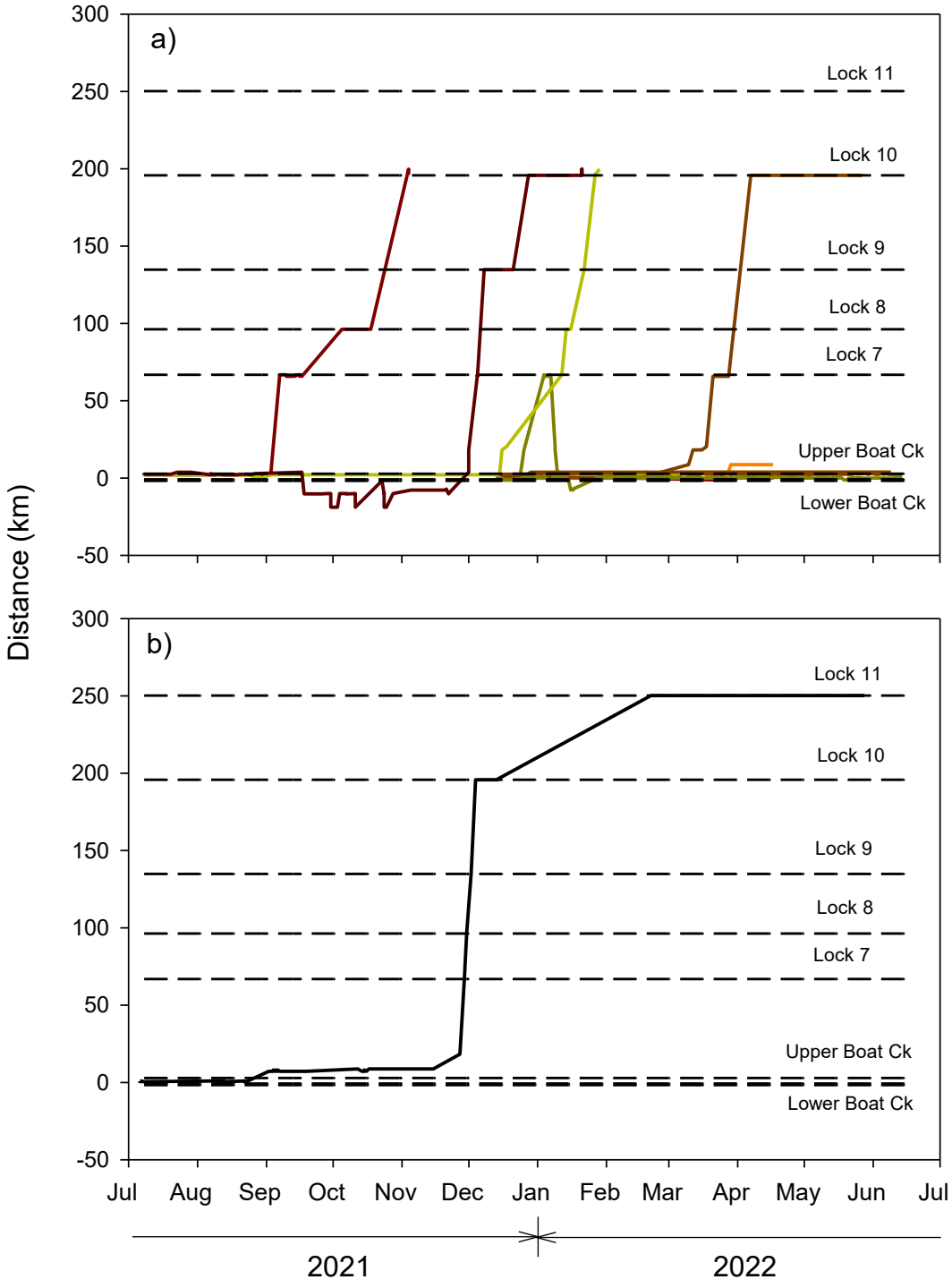

Figure 6. Movement patterns of acoustically tagged a) golden perch and b) silver perch in Boat Creek between July 2021 – June 2022. Dashed lines represent the lower and upper ends of Boat Creek as well as key flow regulating structures on the River Murray.

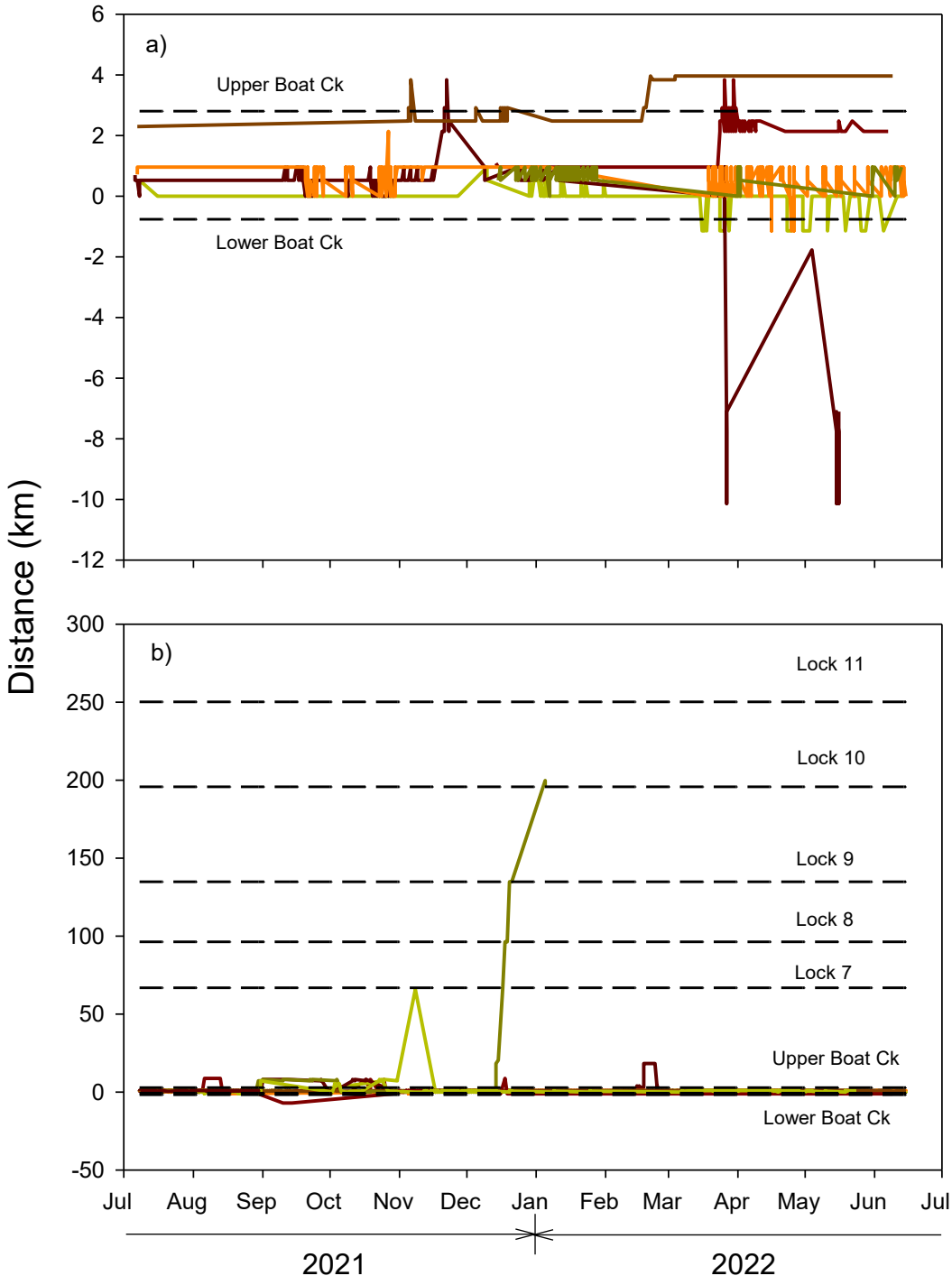

Figure 7. Movement patterns of acoustically tagged a) Murray cod and b) common carp in Boat Creek between July 2021 – June 2022. Dashed lines represent the lower and upper ends of Boat Creek as well as key flow regulating structures on the River Murray.

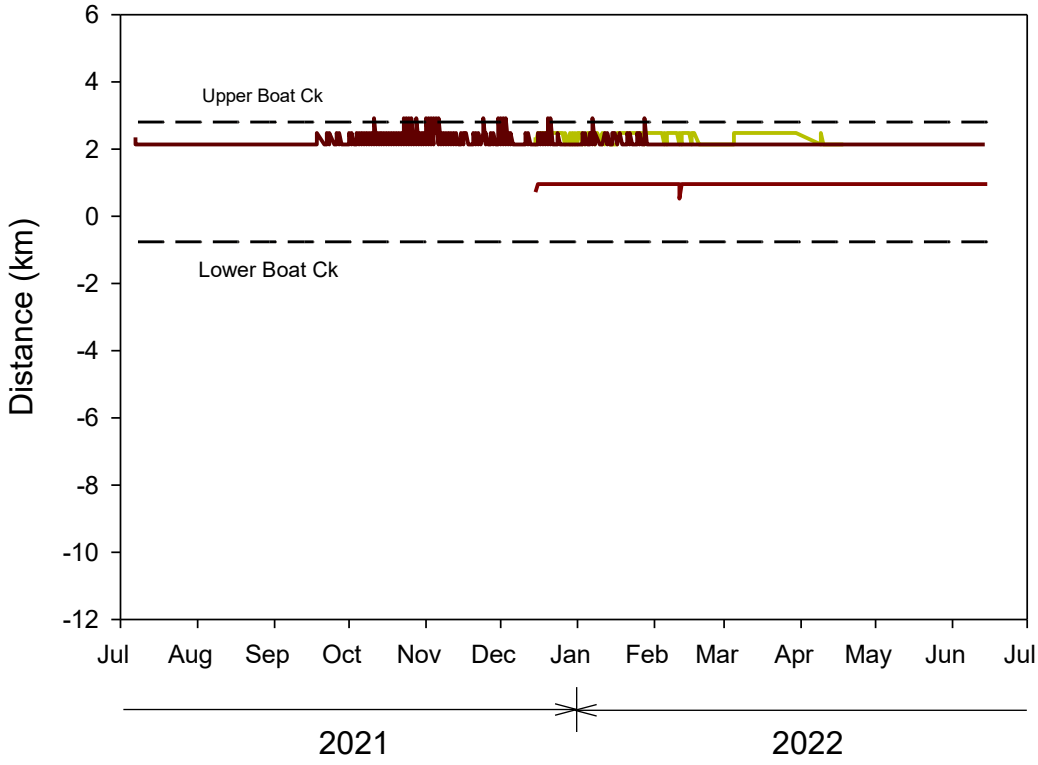

Figure 8. Movement patterns of acoustically tagged freshwater catfish in Boat Creek between July 2021 – June 2022. Dashed lines represent the lower and upper ends of Boat Creek as well as key flow regulating structures on the River Murray.

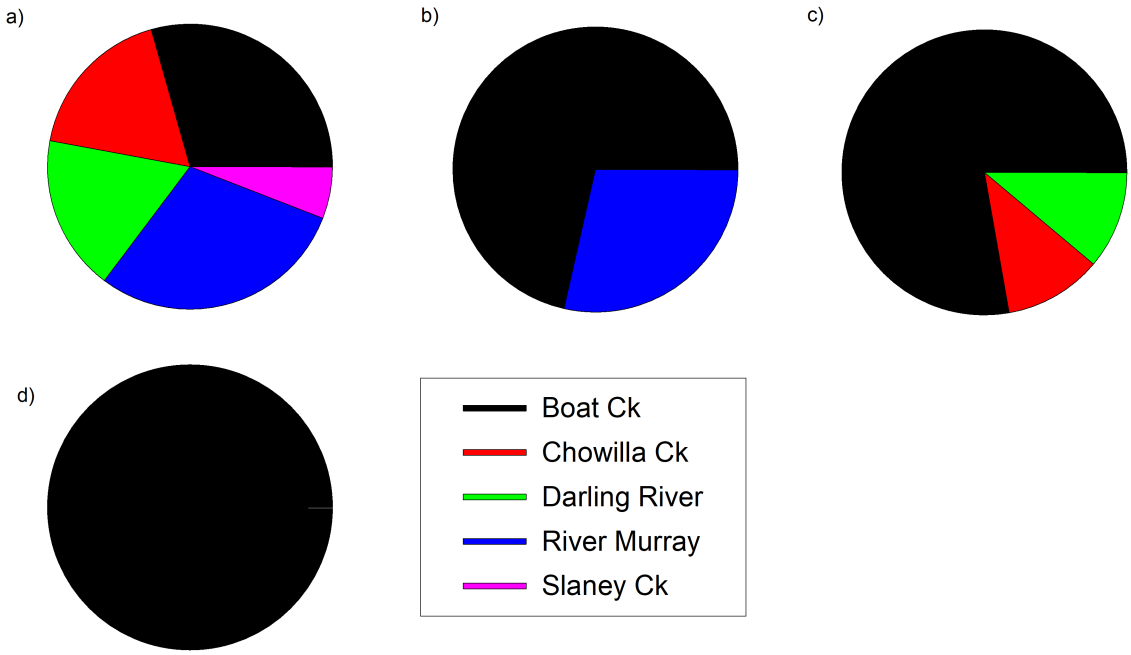

Figure 9. Final reach destination recorded for tagged a) golden perch, b) Murray cod, c) common carp and d) freshwater catfish from Boat Creek between July 2021 – June 2022.

Fish passage

There were 23 instances of fish passage through the Boat Creek Bridge in either an upstream or downstream direction (Table 8). Instances of upstream and downstream passage were detected for Murray cod, golden perch and common carp, a single upstream movement for silver perch, and no passage events for freshwater catfish. Passage events occurred over a broad period from August 2021–March 2022, which included the period of regulator operation and non-operation (Figure 10). Individual upstream passage events occurred when water levels immediately upstream of the Chowilla Regulator were 16.87–19.69 m AHD and downstream passage events when water levels were 17.40–19.57 m AHD. Outside of regulator operations, passage events occurred when water levels were ≥16.87 m AHD at the Chowilla Regulator and were associated with QSA of ≥16,800 ML.day^{-1}.

Table 8. Individual details, daily discharge (QSA, ML.day^{-1}) and water levels at the Chowilla Regulator (m, AHD) at the time of upstream and downstream movement of tagged fish through the Boat Creek Bridge. * indicates individuals that undertook both upstream and downstream movements.

Species	Fish ID	Fish Length (mm)	Date	Discharge	Chowilla WL
Upstream passage					
Golden perch	60359*	408	1/12/2021	26,709	17.464
	60334	473	26/12/2021	37,164	17.941
	60336	390	12/02/2022	26,208	17.363
	60344	480	21/02/2022	27,191	17.518
	60338	400	27/03/2022	17,803	16.911
Silver perch	60355	382	25/08/2021	22,566	18.076
Murray cod	60356	470	27/10/2021	30,257	19.443
	60354*	398	20/11/2021	30,780	18.016
	60332	415	24/03/2022	16,843	16.884
Common carp	60339*	446	05/08/2021 11/10/2021 22/10/2021 26/10/2021	13,341 29,741 30,257 30,257	16.869 19.592 19.541 19.492
	60340*	318	27/09/2021	29,407	19.480
	60341*	450	31/08/2021 5/10/2021 13/02/2022	24,045 29,741 26,208	18.420 19.568 17.360
	60349*	442	29/08/2021 14/12/2021	24,177 27,920	18.319 17.439
	60350*	408	6/11/2021 17/12/2021	33,121 29,741	18.718 17.619
	60351*	685	31/08/2021 2/10/2021 17/10/2021	24,045 29,741 28,962	18.420 19.558 19.531

Table 8 continued.

Species	Fish ID	Fish Length (mm)	Date	QSA (ML.Day)	Chowilla WL (m)
Downstream passage					
Golden perch	60359*	408	17/09/2021	21,361	19.338
Murray cod	60354*	398	09/12/2021	27,303	17.391
Common carp	60339*	446	13/08/2021 19/10/2021 22/10/2021 26/10/2021	20,794 29,417 30,257 30,257	17.490 19.528 19.541 19.492
	60340*	318	17/11/2021	33,306	18.244
	60341*	450	29/09/2021 27/10/2021 24/02/2022	30,554 30,257 28,512	19.510 19.443 17.569
	60349*	442	05/12/2021	27,120	17.397
	60350*	408	06/11/2021 19/12/2021	33,121 32,753	18.718 17.660
	60351*	685	24/09/2021 6/10/2021 26/10/2021	28,116 31,132 30,257	19.443 19.573 19.492

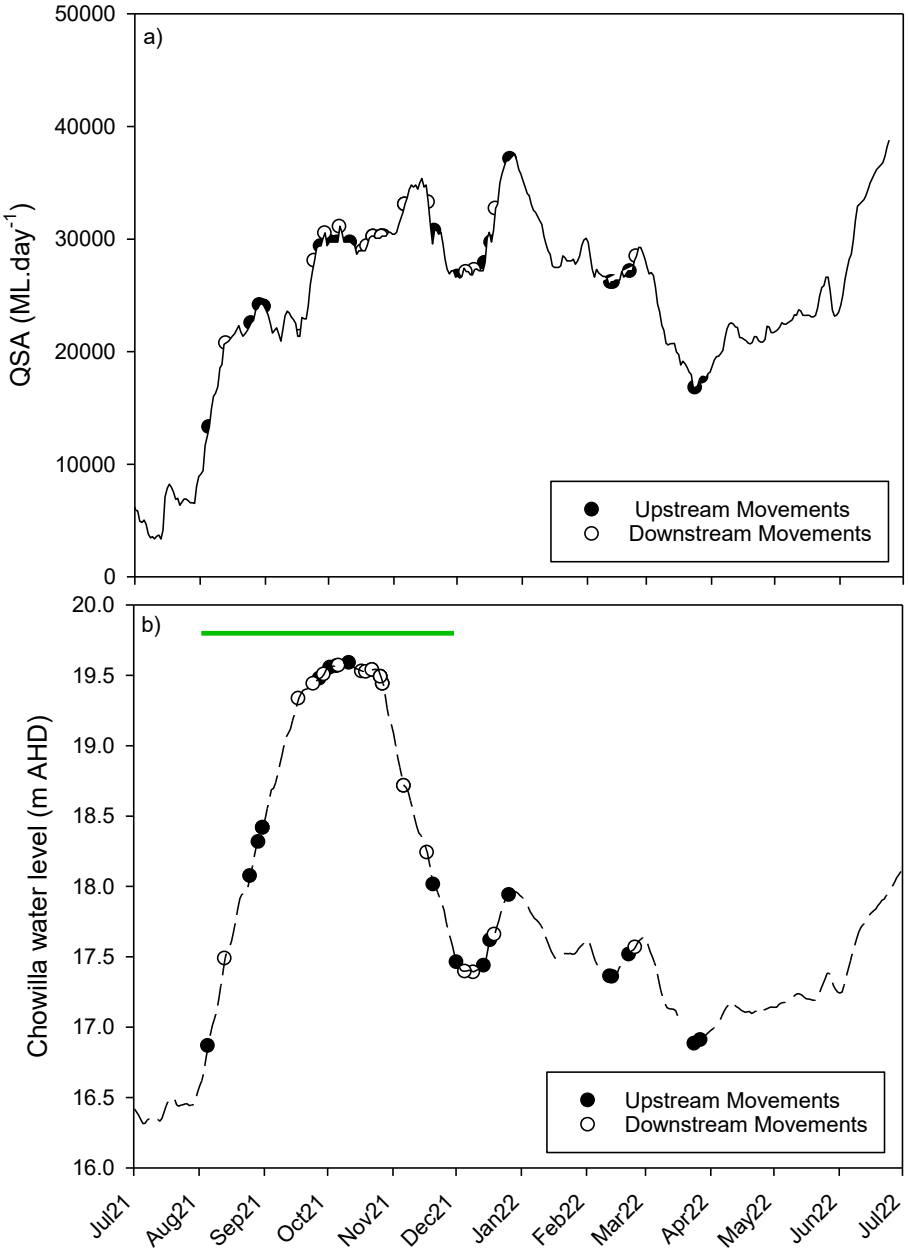

Figure 10. Upstream (*closed circles*) and downstream movements (*open circles*) of tagged fish at the Boat Creek bridge barrier in relation to a) QSA (ML.day^{-1}) and b) Chowilla water levels (m, AHD). Green line on plot b indicates period of regulator operation in 2021.

3.4. Murray cod radio-telemetry

Infrastructure maintenance

Following the major upgrade of two towers in 2020/21, a further three logging stations: 1) the junction of Salt and Slaney creeks, 2) the junction of Salt and Punkah creeks, and 3) the junction of the River Murray and Swifty's Creek were fully upgraded in 2021/22 (Table 9). This included: 1) replacement of existing 80 W solar panels with 160 W panels; 2) replacement of original solar regulators with Victron energy-smart bluetooth controlled regulators; and 3) replacement of AGM batteries with 105ah lithium batteries (Table 9). These upgrades increased power supply and reduced risk of "down time", while the lithium batteries also have a far greater estimated life span (>10 years) than the previous AGM batteries (3–4 years). Additionally in 2022, minor upgrades were also performed on all logging stations which consisted of replacing the antenna coaxial cables with new cables and connectors (Table 9). This was done to ensure connection from the aerial to the ATS R4500C receiver was not compromised, as the old cables were beginning to degrade. On all logging stations the 3G antenna were maintained but will require replacement in the future.

Between October and December 2022, key components (i.e. receiver, batteries, regulators, KLK, solar panels, etc.) of six of the nine logging stations were removed due to rising water levels and major flooding in the lower River Murray. These will be returned in 2023. The Punkah, Bank K and Boat Creek logging stations remained operative during this time.

Table 9. Status and details of completed and potential future upgrades at nine radio telemetry logging stations in Chowilla as of December 2022.

Logging Station	Status	Recent upgrades	Potential future upgrades
Murray	Temporarily removed	160w solar panel 105ah lithium battery Solar regulator Coaxial cables and connectors	Replace 3g modem to 4g or 5g
Regulator	Temporarily removed	Coaxial cables and connectors	Replace 3g modem to 4g or 5g
Boat Creek	Operational	Coaxial cables and connectors	160w solar panel 105ah lithium battery Solar regulator Replace 3g modem to 4g or 5g
Pipeclay Creek	Temporarily removed	Coaxial cables and connectors	160w solar panel 105ah lithium battery Solar regulator Replace 3g modem to 4g or 5g
Slaney Creek	Temporarily removed	160w solar panel 105ah lithium battery Solar regulator Coaxial cables and connectors	Replace 3g modem to 4g or 5g
Salt Creek	Temporarily removed	160w solar panel 105ah lithium battery Solar regulator Coaxial cables and connectors	Replace 3g modem to 4g or 5g
Swifty's Creek	Temporarily removed	160w solar panel 105ah lithium battery Solar regulator Coaxial cables and connectors	Replace 3g modem to 4g or 5g
Punkah Creek	Operational	160w solar panel 105ah lithium battery Solar regulator Coaxial cables and connectors	Replace 3g modem to 4g or 5g
Bank K	Operational	Coaxial cables and connectors	160w solar panel 105ah lithium battery Solar regulator Replace 3g modem to 4g or 5g

3.4.1. Status of tagged Murray cod population

In 2022, a total of 46 radio-tagged Murray cod were detected within the Chowilla system and adjacent River Murray via manual or remote tracking (Figure 11). Of these, 15 have tags that were expected to expire in 2022, while the remainder have tags that are due to expire between 2025 and 2040. Post 2022, the tagged population will be reduced to approximately 30 individuals.

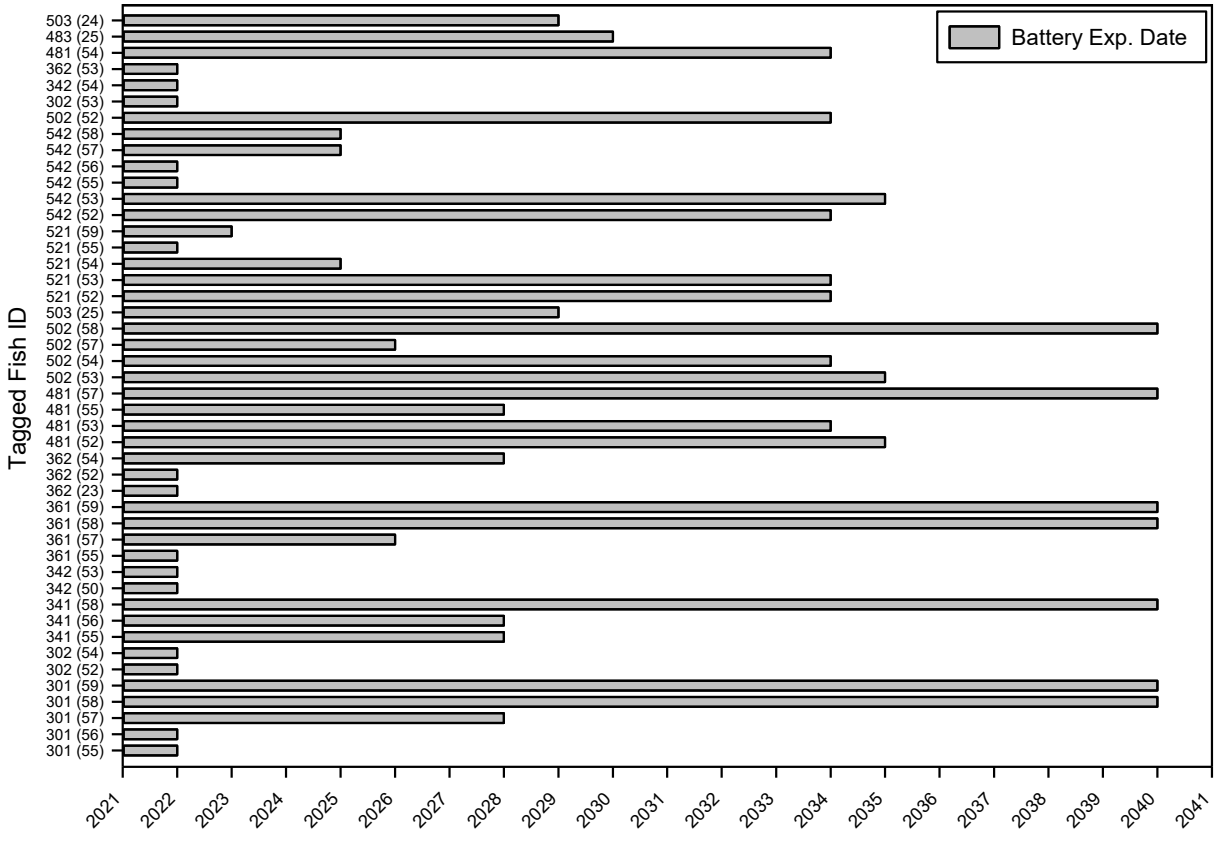

Figure 11. The expected battery-life of radio-tags fitted to 46 ‘active’ tagged Murray cod within Chowilla as of December 2022. The red bar indicates a radio-tag fish that is still operational but has not been actively tracked since 2020.

4. DISCUSSION

The Chowilla system supports a suite of large-bodied native fishes, including a regionally important population of Murray cod. Maintaining and improving this Murray cod population is a key objective of management of the Chowilla Icon Site. As such, an understanding of movement and population dynamics, particularly in relation to operation of the Chowilla Regulator and associated infrastructure, is required to inform adaptive management.

As an *Icon Site* under the Murray-Darling Basin Authority's (MDBA) *The Living Murray Program* (TLM) (MDBA 2016), Chowilla has been subject of a long-term fish condition monitoring program (e.g., Fredberg et al. 2022) and an associated intervention monitoring program that supports targeted investigations (e.g., Bice et al. 2023) to inform specific interventions and adaptive management. This report presents the fish intervention monitoring program for 2021/22, which included four specific but related components, namely: 1) assessing reproductive variance and effective population size of Murray cod; 2) trialing the use of epigenetic ageing to produce an age structure for Murray cod from Chowilla; 3) assessing fish movement, habitat use and passage within Boat Creek; and 4) performing maintenance on radio-telemetry infrastructure and summarising the status of the tagged Murray cod population. The results of each component are discussed below, followed by commentary on potential future investigations.

4.1. Murray cod kinship, breeding output and effective population size

This study has continued to build upon an existing dataset on Murray cod genetics from Chowilla that commenced with sample collection in 2017 (Gibbs et al. 2020). The results provide further evidence of Murray cod mating systems, including the presence of within-seasonal polygamy, between-seasonal polygamy, between-season monogamy, and reproductive skew within breeding populations (Couch et al. 2020, O'Dwyer 2022). Ultimately, within the Chowilla region, Murray cod appear to exhibit mixed mating strategies, as they exhibit in other regions of the MDB (Couch et al. 2020).

The estimated number of breeding adults that contributed to cohorts (by spawning seasoning) and reproductive skew (the number of offspring contributed by each breeding adult) varied considerably among years. For instance, more full-siblings were detected in 2021 compared to earlier cohorts, while more half-siblings were detected in the 2018 cohort compared to other cohorts. Overall, patterns of sibship and parentage influence estimates of effective population size.

Per cohort effective population size estimates, which indicate the number of effective breeders per breeding cycle, varied among years. These estimates, however, are sensitive to the relative percentage of larvae in the offspring sample compared to other nonadult life stages, with greater proportions of larvae resulting in a greater level of sibling relationship and lower effective population size. Nonetheless, the 2017 cohort appeared to be the most genetically diverse, with the largest effective population size estimate (LD Ne = 113.9, Colony Ne = 128), even when the analysis was based only on larvae (LD Ne = 57.6, Colony Ne = 61). The 2021 cohort appeared to be associated with the lowest effective population size (LD Ne = 49.2, Colony Ne = 44), yet when only larvae were considered (LD Ne = 48, Colony Ne = 43), estimates were comparable to most previous years.

Although the per cohort effective population size is likely to underestimate the true number of breeders, due to incomplete sampling of the larval cohort, the cohort effective population size in any given year is typically 6–15x lower than the adult effective population size estimate, suggesting that not all adults in the population may breed in a given year. Per cohort effective population size estimates can be used to detect signals of population decline over time (e.g., Luikart *et al.* 2020). Continued monitoring of the Chowilla Murray cod population will be important to understand the trajectory of effective population size in the population and whether there is a trend of declining or increasing genetic diversity in the population or just annual fluctuations that are either stochastic or in response to environmental conditions.

The integration of sequenced offspring from the Lindsay-Mullaroo system, with those from Chowilla, provided evidence of shared sibling pairs and hence movement of fish between these systems. This may be promoted by the movement and spawning of reproductively mature individuals or the movement of one of a sibling pair between systems. Movements of radio-tagged tagged fish between Chowilla and Lindsay-Mullaroo has been previously observed (SARDI unpublished data; Arthur Rylah Institute unpublished data) lending support to the first mechanism. Together, kinship analysis and radio-telemetry indicate connectivity between Murray cod populations in two perennial lotic systems in the largely lentic lower River Murray.

4.2. Epigenetic ageing

For our trial of the non-lethal epigenetic ageing method, despite following the published protocols, we were unable to obtain six of the 26 CpG sites required to fully implement the epigenetic clock of Mayne et al. (2021). Whilst we were able to generate predicted ages for Murray cod, age

estimates were based on a reduced set of clock sites rendering predicted ages unreliable. Issues with limited CpG sites were confounded further by a lack of older (> 1 year) Murray cod individuals in our dataset, making rigorous assessment of age estimation difficult.

To address issues of limited CpG sites and lack of known-age individuals, we expanded our Murray cod dataset to also include: 1) sequencing read data for Murray cod and Mary River cod (*Maccullochella mariensis*) individuals of known age from Mayne *et al.* (2021) and sequencing read data for golden perch (*Macquaria ambigua*) individuals of known age (LaTrobe University unpublished data); and 2) all CpG sites that were present in the sequence data (not just the published CpG from Mayne et al. 2021). Using this expanded dataset, we were able to develop a new epigenetic clock, which resulted in significantly improved, albeit low confidence, age prediction (r^2cv = 0.41). Most of the predictive power appeared to be driven by the inclusion of golden perch, most likely because this increased the range of ages in the dataset (because all the fish older than 12 years in the dataset were golden perch). A larger sample of known-age individuals, and particularly individuals from older age classes, will be important for more rigorous age prediction.

In summary, our findings suggest that the non-lethal epigenetic ageing method shows promise but requires further investigation with a larger number of samples, including samples of known age. It will also be important to understand how issues with sample quality may impact on age estimates. The fact that golden perch appear to show similar methylation-age relationships at the same set of CpG sites as Murray cod and Mary River cod suggests that these amplicons could be trialled more widely across percichthyids, with scope to develop an epigenetic clock that could be applied with some generality across this taxonomic group.

4.3. Fish movement and habitat use in Boat Creek

Monitoring of large-bodied fish movement in Boat Creek and the broader Chowilla system occurred during a period of variable hydrology (QSA range = 3,359–37,567 ML.day^{-1}) that also included an operation of the Chowilla Regulator from August–December 2021. The data obtained provides insights on: 1) differences in movement patterns among species, 2) use of Boat Creek as a habitat, 3) passage through the Boat Creek Bridge and 4) use of the floodplain lakes of Chowilla.

Patterns of movement of the five tagged species were consistent with previous studies and suggest a general continuum of spatial-scale of movement with golden perch and silver perch being the most mobile, followed by common carp, Murray cod and freshwater catfish (Koster et al. 2014, Zampatti et al. 2018, Koster et al. 2021, Thiem et al. 2022). The sole silver perch tagged, several golden perch and an individual common carp traveled distances of ≥200 km upstream and transited to the Darling or mid-Murray rivers. Excluding these large-scale movements, golden perch also exhibited movements between Boat Creek and other reaches of the Chowilla system (e.g., Chowilla and Slaney creeks) and the adjacent River Murray. No golden perch visited floodplain lakes. At the cessation of tracking, however, most golden perch (53%) remained within Chowilla.

Common carp also exhibited frequent local-scale movements within Chowilla among Boat Creek, Chowilla Creek, and the adjacent River Murray. They also exhibited a high frequency of use of floodplain lakes during the regulator operation. In September and October 2021, over half of the tagged common carp (*n* = 5) entered and exited Lake Limbra via Hancock Creek, while an additional fish was detected in Coombool Swamp. When inundated, lakes on the Chowilla floodplain represent favourable spawning and nursery habitats for common carp (sensu Stuart and Jones 2006) and the results of this study provide further evidence that common carp within Chowilla readily move to these lakes when inundated during the spawning season (September–March).

Murray cod and freshwater catfish exhibited more restricted movements relative to the other species. Most Murray cod remained within Boat Creek for much of the study, but several individuals made regular small-scale (<10 km) movements within Boat Creek or between Boat Creek and Chowilla Creek, and the adjacent River Murray. This general pattern of local movement among favoured lotic habitats is consistent with previous studies in the system (Fredberg et al. 2019). Throughout the study, the small number of freshwater catfish detected (*n* = 3) remained within Boat Creek and <1 km from tagging locations. This is the first tracking data derived from freshwater catfish in the South Australian River Murray and suggests very restricted movement consistent with the few other studies that have investigated freshwater catfish movement in the southern MDB (e.g. Koster et al. 2014).

Passage through the Boat Creek Bridge was documented for golden perch, silver perch, Murray cod and common carp, and occurred both during and outside of periods of regulator operation.

When water levels downstream of the Boat Creek bridge are elevated, headloss and water velocity through the bridge are reduced and passage of large-bodied fishes appears unimpeded. Instances of upstream passage of golden perch (400 mm TL), Murray cod (415 mm TL) and common carp (446 mm FL) occurred when water level upstream of the Chowilla Regulator was ~16.9 m AHD, including when the regulator was not in operation and QSA was ~17,000 ML.day^{-1}. If a water level of 17 m AHD at the Chowilla Regulator is taken as a level above which upstream passage through the Boat Creek Bridge may be facilitated for adult large-bodied fish, from July 2012–June 2022, upstream passage through the bridge would have been supported in seven of ten 'water years' (July–June) and ~25% of total days, regardless of regulator operation. During extended periods of low flow (e.g., July 2002–June 2010), however, passage is unlikely to be facilitated based just on flow in the river. Nonetheless, future extended periods of low flow are likely to include regulator operations when passage would be facilitated. Downstream passage events were less frequent and documented when water levels were generally >17.4 m.

While the passage of large-bodied fishes is likely facilitated with some regularity through the Boat Creek Bridge, the upstream passage of small-bodied species through the structure remains unknown. Due to their weaker swimming abilities, it is likely that passage is facilitated less frequently and possibly only when there is minimal head difference across the structure. This likely includes most regulator operations. While free movement through the riverscape is required for all fishes, the movement of small-bodied fishes through the Boast Creek Bridge is not considered critical to their life histories.

4.4. Maintenance of telemetry infrastructure and tag status of tagged Murray cod

The spatial ecology of Murray cod in Chowilla and adjacent River Murray has been a focus of research since 2007, with specific studies identifying key habitats within the system, temporal and flow-related patterns of movement, and the influence of the Chowilla Regulator on movement and habitat characteristics (Leigh and Zampatti 2013, Zampatti et al. 2016, Fredberg et al. 2019). Most studies have used radiotelemetry and been supported by a series of nine telemetered logging stations (ATS radio receiver/loggers) located on major tributaries of Chowilla Creek, at the junction of Chowilla Creek and the River Murray and on the Chowilla Regulator. A total of >100 Murray cod have been tagged and their movements monitored over this time. To ensure system functionality and enable continued investigations of Murray cod in the region, there is a need to

provide periodic maintenance of radio-telemetry infrastructure and understand the status of the radio-tagged Murray cod population.

As of late 2020, five of the nine remote logging stations at Chowilla, were offline or operating intermittently due to issues with power supply caused by battery degradation and insufficient solar input. Furthermore, all units required software upgrades. In 2020/2021, software was upgraded in the KLK3000 units in all logging towers, while two loggers were extensively upgraded. In 2021/22, the remaining three towers with power supply issues were extensively upgraded, including solar panels, regulators, and battery replacement. Furthermore, the coaxial cables and connectors were replaced on all nine towers. As of winter 2022, all remote logging stations were performing satisfactorily. This included the four loggers yet to undergo full refurbishment. Extensive flooding in the lower River Murray in late 2022, however, necessitated the removal of critical componentry from all but three logging stations. The reinstatement of these logging stations is planned for late-2023.

Based on estimated battery life, in 2023, ≤31 Murray cod still have active radio tags. Of these, most are due to expire in 2026 and beyond. Maintaining a tagged population of ≥50 individuals is likely required to support scientifically robust investigations of movement and as such, there is a need to tag more individuals. The movement and habitat use of Murray cod within Chowilla, as well as recruitment and general population dynamics, remain areas of interest due to ongoing operation of the Chowilla Regulator and proposed manipulation of water levels in Lock 6, which will influence head differential across the Chowilla system, and subsequently discharge, hydrodynamics and connectivity. Acoustic telemetry represents an alternative approach to radio-telemetry for studying fish movement and presents some notable advantages, including greater spatial resolution of monitoring. As such, to support investigation of Murray cod movement into the future, we recommend a gradual transition to acoustic telemetry, including tagging of fish. Nonetheless, we also recommend continued base level maintenance of radio-telemetry infrastructure to support continued monitoring of individuals with long-term (estimated battery life up to 2040) radio-tags.

5. CONCLUSIONS AND RECOMMENDATIONS

In 2021/22, we continued investigations of reproduction and populations dynamics of Murray cod using genetic approaches, and conducted an additional study of habitat use, movement and passage of large-bodied fishes within Boat Creek. We also conducted critical maintenance on radio-telemetry infrastructure. The results of the current project highlight several future investigations and tasks to build upon past studies and further inform management of Chowilla, particularly regarding the ecology of Murray cod. As such, we recommend the following:

- Continuing investigations of Murray cod adult contribution, movement and effective population size using genetic approaches. The existing Chowilla Fish Condition Monitoring program provides a means for continued collection of genetic samples. Interpretive power will increase with increasing sampling period and sample size, providing greater insight on the influence of management and river conditions on reproduction/recruitment and connectivity among Chowilla and Lindsay-Mullaroo. This may be increasingly important given proposal to build and operation flow regulating infrastructure in the Lindsay-Mullaroo system similar to Chowilla.
- Further epigenetic ageing of Murray cod is not recommended until the technique is better refined.
- Passage through the Boat Creek bridge was documented for adult golden perch, silver perch, Murray cod and common carp when water levels upstream of the Chowilla Regulator were >17 m AHD. This corresponds to periods when QSA is >17,000 ML.day^{-1} and during most regulator operations. As such, passage is likely provided past this structure, at times, in most years. The passage of small-bodied fishes was not studied but is likely facilitated during most regulator operations. As such, construction of a fishway at this site is a low priority.
- Following Intervention Monitoring in 2021/22, all radio-telemetry towers in immediate need of maintenance will have been upgraded with new componentry. Post-flood re-instatement of receiver componentry in all towers will likely occur late-2023.
- Continued investigations of Murray cod movement and habitat use in Chowilla and the adjacent River Murray will necessitate the tagging of further individuals. We recommend continuation of tracking of existing radio-tagged individuals while tags have remaining battery, but to transition to acoustic telemetry for newly tagged fish in coming years. This

will support future investigations of Murray cod movement, but also other species of conservation concern (e.g., freshwater catfish).

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