Inland Waters &
Catchment Ecology

Chowilla Icon Site Fish Intervention Monitoring 2022/23

C. M. Bice[1], J. F. Fredberg[1], K. A. Harrisson[2], N. P. Murphy[2], J. O'Dwyer[3], B. G. Fanson[4], S. Leigh[5] and B. P. Zampatti[6]

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Report to the Department for Environment and Water

Government of South Australia
Department of Primary Industries and Regions

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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 as such 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.

In 2022/23, the primary objectives of this project were to:

1) Capture Murray cod larvae and young-of-year (YOY) derived from spawning in 2022 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; and
2) Undertake a meta-analysis of Murray cod movement data collected from 2008–2023 to assess:
    a. General patterns of residency and movement within Chowilla;
    b. Rates and environmental drivers of local Chowilla-scale, Chowilla–Murray and long-distance River Murray movements; and

c. Provide commentary on implications for management and future investigations of Murray cod movement and habitat use.

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 2017–2023 (*n* = 328) 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 were 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 region 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 2017–2022 were estimated to be derived from 269 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 269 adults identified. Estimated values of effective population size and the ratio of number of breeding parents identified to the number of offspring sequenced, varied annually (the sample of individuals derived from spawning in 2022 was too small to enable estimation of effective cohort population size). 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 this was 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 movement meta-analysis

A dataset comprising the movement of 108 Murray cod radio-tagged at Chowilla over the period 2008–2023 was analysed to assess patterns of occupancy and movement, and key drivers of movement. Most individuals (~80%) moved over a limited linear extent (<40 km) and exhibited high fidelity for lotic reaches within Chowilla (e. g. Slaney, Pipeclay and Chowilla creeks) and movements between these reaches. For fish with long movement histories (7–13 years) these movement patterns showed a high level of annual repeatability. Movements to the adjacent River Murray were also common with over half of the tagged fish making at least one movement to the River Murray during the study period. Long-distance movements (>40 km) in the River Murray were also observed but were less common (*n* = 16, 15% of tagged fish).

Key drivers of local Chowilla-scale movements included time of year, River Murray discharge and Chowilla water levels, with higher movement rates occurring during August–October in association with elevated river discharge and elevated Chowilla water levels. While there were no significant drivers of Chowilla–Murray movements, once in the River Murray, long-distance upstream movements were similarly related to time of year (August–October) and elevated River Murray discharge. The influence of Chowilla water levels was most pronounced during river discharge <30,000 $ML.d^{-1}$, indicating that operation of the regulator increases rates of local movement. Nonetheless, the influence of such an effect on populations dynamics remains unclear. Operation of the regulator, however, during peak periods of movement in August–October may impede movements between Chowilla Creek and the River Murray.

The results of this study reinforce the importance of lotic habitats in Chowilla for Murray cod and connectivity between these habitats and with the River Murray. Operation of the Chowilla Regulator and future water level manipulation in the Lock 6 weir pool may influence Murray cod habitat and movement. As such, continued investigation of Murray cod movement, alongside monitoring of population demographics, is recommended to inform adaptive management of the Chowilla Icon Site and broader lower River Murray. Such investigations will be best supported by continuation of radio-telemetry monitoring of movement, but a gradual transition toward acoustic telemetry.

Keywords: Murray cod, genetics, kinship, movement, 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 degraded by the impacts of river regulation. In-channel habitats have been fragmented by barriers to flow and fish movement, while decreased flooding frequency and salinisation impact the condition and recruitment of long-lived floodplain eucalypts. Nevertheless, the site is now the focus of substantial ecological restoration effort. Several in-channel barriers to flow and fish movement have been remediated through regulator upgrades and fishway construction (e.g. Slaney and Pipeclay weirs). In 2014, following construction of the Chowilla Creek Regulator and ancillary structures, large-scale engineered floodplain inundation commenced 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 risks include: 1) fragmentation of habitats and obstruction of movement; and 2) alteration of stream hydraulics (e.g. reduced water velocities). These mechanisms may act in unison to impact 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 2019). To better understand the ecology of Murray cod and other fish species at Chowilla, including responses to regulator operation and other management interventions, an *intervention monitoring* program supports targeted investigations to inform adaptive management. This report presents the fish intervention monitoring program for 2022/23, which included two 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 demographics (e.g. age structure), as well as reproductive characteristics such as 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 2017–2022 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. 2023a and b). 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 Condition Monitoring and provide greater insight on Murray cod reproduction and population dynamics in the Chowilla region, including the influence of system management.

Murray cod movement meta-analysis

The movement of Murray cod in the Chowilla system and the adjacent River Murray has been a focus of research since 2008 (Leigh and Zampatti 2009). Studies have primarily used radio-telemetry and involved the tagging of >100 individual Murray cod and tracking of movement both manually and remotely using nine telemetry 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 Chowilla Murray cod radio-telemetry dataset now extends over 15 years (2008–2023) that comprises extreme hydrological variability (drought and flood) and consequent floodplain inundation, including natural and engineered events. As such, the dataset presents an opportunity to use quantitative modelling approaches to investigate several aspects of the spatial ecology of Murray cod at Chowilla with regards to hydrology and site management. This information can inform future adaptive management of Murray cod at Chowilla and across the broader region.

1.2. Objectives

In 2022/23, the primary objectives of this project were to:

1) Capture Murray cod larvae and young-of-year (YOY) derived from spawning in 2022 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; and
2) Undertake a meta-analysis of Murray cod movement data collected from 2008–2023 to assess:
    a. General patterns of residency and movement within Chowilla;
    b. Rates and environmental drivers of local Chowilla-scale, Chowilla–Murray and long-distance River Murray movements; and

c. Provide commentary on implications for management and future investigations of Murray cod movement and habitat use.

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).

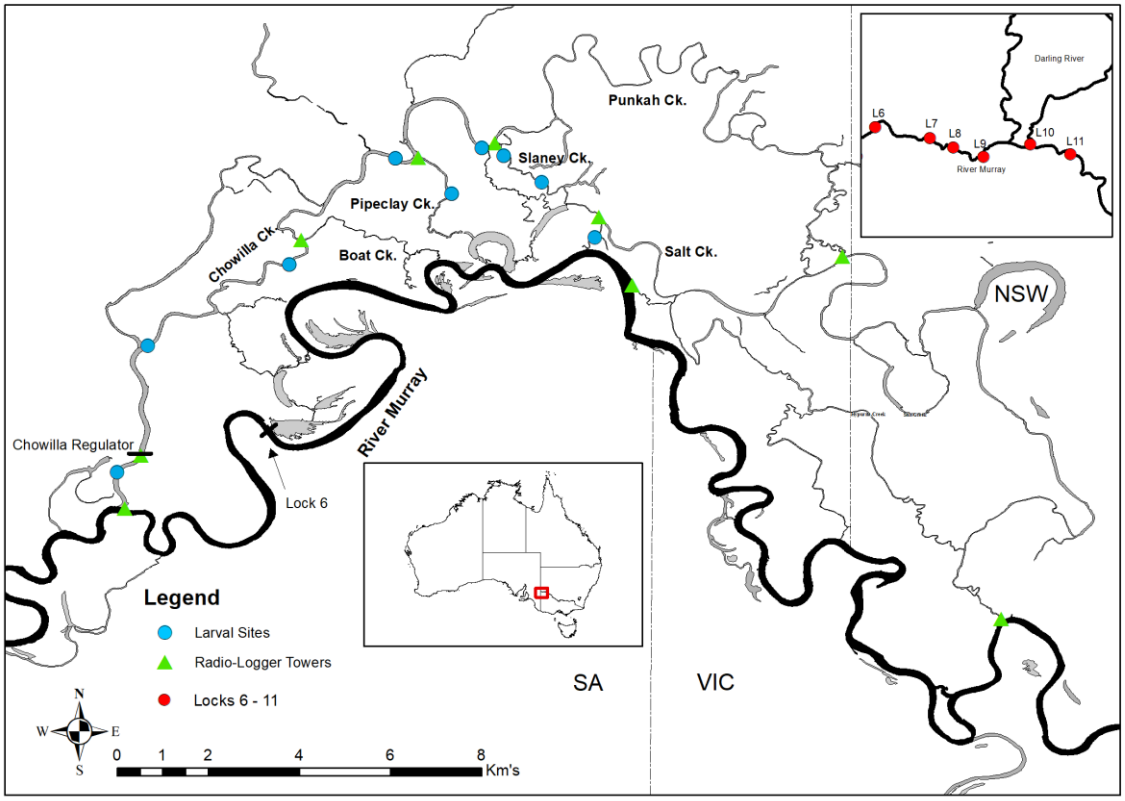

Figure 1. Map of the Chowilla Anabranch System on the River Murray, South Australia, depicting nine sites sampled for Murray cod larvae (blue circles) and nine fixed radio-telemetry stations (logger towers) used to gather data for Murray cod movement from 2007–2023 (green triangles). Inset top right depicts the lower River Murray from Lock 6–10 and position of weirs and associated fishways and PIT readers (red circles).

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 2017–2022 (see Gibbs et al. 2020, Bice et al. 2023a and b), while in 2022/23, sampling of Murray cod derived from spawning in spring 2022 occurred via two approaches: 1) larval sampling in spring 2022; and 2) sampling of YOY Murray cod in autumn 2023 during annual condition monitoring electrofishing surveys (Fredberg et al. 2024). In addition, to supplement larval 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 2018–2023 were also included to investigate movement of fish between Chowilla and Lindsay-Mullaroo.

In 2017, 2018, 2020, 2021 and 2022 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). Most sites were sampled consistently across years, except for Chowilla upstream of Monoman and downstream of the regulator, which could not be sampled in 2022 due to limited access because of flood. These sites were replaced by Little Slaney Creek and Slaney Creek mid (Figure 1; Table 1).

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, 2021 and 2022 including the number of sampling events for drift nets and light traps.

	2017		2018		2020		2021		2022	
Site name	**Drift net**	**Light trap**	**Drift net**	**Light trap**	**Drift net**	**Light trap**	**Drift net**	**Light trap**	**Drift net**	**Light trap**
Little Slaney Creek	-	-	-	-	-	-	-	-	*N* = 2	*N* = 4
Slaney Creek mid	-	-	-	-	-	-	-	-	*N* = 2	*N* = 4
Slaney Creek	-	*N* = 5	-	*N* = 3	*N* = 2	*N* = 3	*N* = 2	*N* = 3	*N* = 2	*N* = 4
Chowilla downstream Slaney	*N* = 6	*N* = 6	*N* = 5	*N* = 6	*N* = 2	*N* = 4	*N* = 2	*N* = 4	*N* = 2	*N* = 4
Pipeclay Creek	-	-	-	-	*N* = 2	*N* = 4	*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	*N* = 1	*N* = 4
Chowilla downstream Boat	*N* = 6	*N* = 6	*N* = 5	*N* = 6	*N* = 1	*N* = 4	*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	*N* = 1	-
Chowilla downstream Regulator	*N* = 6	*N* = 6	*N* = 5	*N* = 6	*N* = 1	*N* = 3	*N* = 1	*N* = 3	*N* = 1	-

In autumn of 2017–2023, 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 (Fredberg et al. 2023). 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 Lindsay-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. Murray cod movement meta-analysis

Remote radio- and PIT-telemetry

Nine remote logging stations (ATS radio receiver/loggers model No. RC4500C) are located on key creek junctions throughout Chowilla, at the junction of Chowilla Creek and the River Murray (2007 onwards), and on the Chowilla Creek regulator (2014) (Figure 1). Most stations incorporate three Yagi antennas: one facing upstream, one downstream and one in the direction of the tributary. The Chowilla Regulator logging station has two antennas, one facing upstream and one downstream Chowilla Creek. The presence of fish in the vicinity (detection range ≤600 m) of an antenna was recorded automatically as a tag frequency, antenna number, time and signal strength. These data were remotely transmitted to a central database. PIT tagging enabled radio-tagged fish to be detected by PIT tag readers installed in vertical-slot fishways on the Chowilla Creek regulator and in vertical-slot fishways on River Murray locks and weirs (Barrett and Mallen-Cooper 2006). Any fish moving upstream or downstream in the River Murray were identified by interrogating PIT tag reader records from fishways at the Chowilla Creek regulator and Locks No. 1–10.

Radio transmitters implanted into Murray cod were cylindrical 150 MHz, internal transmitters with a 30 cm long (0.7 mm diameter) trailing antenna (Advanced Telemetry Systems (ATS), Insanti, MN, USA). Three sizes of transmitter were used: models F1850, F1855 and F1860, weighing 25, 87 and 150 g in air and having warranted battery lives of 560, 1657 and 3937 days, respectively. Transmitters were fitted with a mortality circuit that activated and produced a distinct signal if the fish (i.e. transmitter) did not move for a period of ≥8 hours. Each radio-tagged fish was also implanted with a passive integrated transponder (PIT) tag (Texas Instruments RI-TRP-REHP half-duplex eco-line glass transponders, 23.1 mm long, 3.85 mm in diameter and weighing 0.6 g in air) to facilitate detection of these fish in fishways fitted with PIT tag readers on the Chowilla Creek

regulator and main channel Locks and Weirs of the River Murray (Barrett and Mallen-Cooper 2006). Plastic tipped dart tags (PDA or PDS, Hallprint, Victor Harbour, SA, Australia) were used to enable external visual identification of radio-tagged fish and reporting of captures by anglers.

Fish capture and surgery

From 2007–2019, 108 Murray cod were captured and implanted with radio-transmitters across multiple tagging events (mean total length [TL] ± SE = 837 ± 21 mm, range = 435–1230 mm TL; mean weight ± SE = 13 ± 0.95 kg, range = 0.9–38 kg; Appendix 1). Fish were captured throughout Chowilla and in the adjacent River Murray main channel using a Smith-Root® 5.0 KVA boat mounted electrofishing unit. Most individuals were captured from Slaney (*n* = 50) and Chowilla creeks (*n* = 22), and the adjacent River Murray (*n* = 16), with smaller numbers tagged in Little Slaney (*n* = 9), Salt (*n* = 7) and Pipeclay creeks (*n* = 4) (Figure 1; Appendix 1).

Following capture, Murray cod were anaesthetised using 1.5 ml of AQUI-S® (Aqui-s, Lower Hutt, New Zealand) per 50 L of river water. Length and weight were recorded and fish were inverted onto a v-shaped cradle. The gills were irrigated throughout the surgery with a 50 % dilute solution of AQUI-S. An incision of 3–4 cm was made through the ventral wall slightly dorsal to the mid-ventral line beginning adjacent the pelvic fin and extending towards the anus. Where possible, the sex of the fish was determined and the transmitter inserted into the abdominal cavity. To ensure fish buoyancy was not compromised transmitter weight was ≤2 % of total body mass.

A shielded-needle technique (Adams et al. 1998) was used to guide the trailing antenna through the lateral body wall posterior to the incision. The incision was closed with two internal and three external sutures. A long-term (2 weeks) antibiotic Baytril® (Bayer Australia, Pymble, NSW, Australia) at a dose of 0.1 mL kg^{-1} was then injected in the dorsal musculature. A PIT tag was inserted in the dorsal musculature forward of the dorsal fin or, in large fish, in the cheek muscle, and a dart tag was positioned between the dorsal pterygiophores. Following recovery, fish were released at their capture location.

Statistical analysis

Classification of Murray Cod Movement data

For the period 2008–2023, a daily movement dataset was created for each tagged fish using both radio- and PIT-telemetry data. Individual profiles started at a fish's tagging location and remained at that location until detected at a new location (receiver or PIT reader), and so on for subsequent detections, until the estimated date of tag expiry or when last detected (whichever is later). A 'move' began if a fish was detected on receivers >2 km apart over a period of seven days and continued until no further movements were detected within a period of seven days. Using these daily profiles, movement events were identified (Figure 2). Based on interrogation of movement profiles and knowledge of previous analyses (Leigh and Zampatti 2013), movement types were defined as:

1) Local Chowilla-scale movements: individual movements of >2 km within the Chowilla system.
2) Chowilla–Murray movements: individual movements that involved a transition between Chowilla and the River Murray (i.e. via the junctions of Chowilla Creek and the River Murray or Swifty's Creek and the River Murray).
3) Long-distance upstream Murray movements: events that began as 'Chowilla–Murray movements' that continued for >40 km upstream in the River Murray.
4) No movement: periods of time when the above criteria were not met were classified as 'non-moving'.

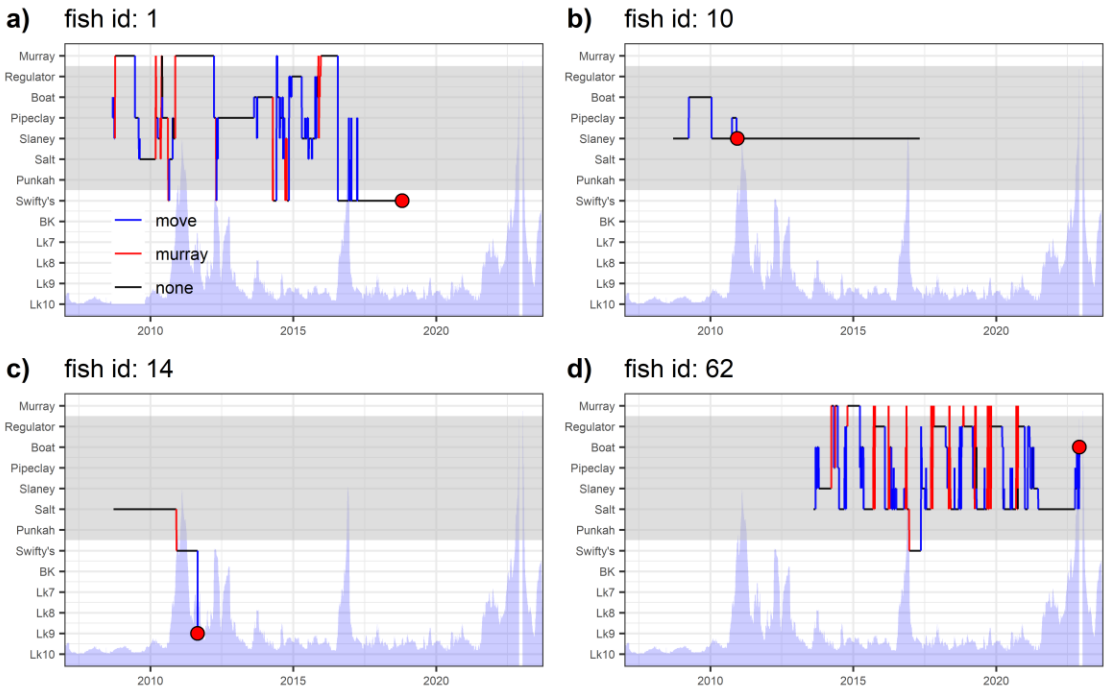

Figure 2. Example of movement profiles of four tagged Murray cod. The black line indicates a non-movement, red line indicates a Chowilla–Murray movement and the blue line indicates a movement (that is not a Chowilla–Murray movement). The red dot indicates last known detection at a receiver/PIT reader. The grey area indicates Chowilla receivers, whilst blue shading is the River Murray hydrograph (QSA). Receivers are ordered by distance from downstream (top y axis) to upstream (bottom of y axis).

Analysis of movement data

These analyses sought to determine the influence of hydrology and operation of the Chowilla Regulator on the defined movement types. Key inputs to the models are River Murray discharge (Flow to South Australia, QSA, ML.d^{-1}) and water levels immediately upstream of the Chowilla Regulator (WQ Station A4261224, Water Data SA). Importantly, the relationship between River Murray discharge and Chowilla water level is different between natural flood events and engineered floodplain inundations, and as such, the differing influences of these events may be determined statistically. Water velocity was not explicitly used as a factor in models as it varies greatly across the system. Instead, the relationship between Chowilla water level and River Murray discharge acts as a proxy for hydraulic conditions within the Chowilla system based on known changes in hydraulics with operation of the regulator (e.g. SARDI unpublished data).

Drivers of local Chowilla-scale movements

All analyses were performed in R V4.2 (R Core Team 2022) using the mgcv package (Wood 2017). Movement, River Murray discharge and Chowilla water level data were first summarised into 7-day time windows to reduce dataset size and assuage temporal correlation. Only movement data from within the Chowilla system was used for this analysis, including data in which a fish initiated a movement within Chowilla but was later detected in the River Murray. A Generalised Additive Mixed Model (GAMM) assumed a binomial distribution (i.e. 1 = moving, 0 = not moving) for all models (Appendix 2). The response variable was whether the fish moved during the 7-day window. The predictor variables depended on the model being tested. All models included a penalised cubic spline for date (date of the start of temporal window) and circular spline week within a year, a random effect of fish id, and a fixed effect for whether the previous 7-day window included a fish movement. Model 0 (M0) was the base model and included no flow metrics. Model 1a (M1a) included Chowilla water level (WL) (m AHD), M1b included change in WL (ΔWL), M2a included Murray discharge (MD) (Flow to South Australia, QSA, $ML.d^{-1}$), and M2b included change in MD (ΔMD). M3a included both WL and MD and finally M3b included ΔWL and ΔMD. Model selection was calculated by using Akaikie Information Criteria (AIC) (Appendix 3).

$$M0 = s(date, cs) + s(day_{year}, bs = cc) + Fish\ ID + moved_{prev}$$

$$M1a = M0 + s(WL, bs = cs)$$

$$M1b = M0 + s(\Delta WL, bs = cs)$$

$$M2a = M0 + s(MD, bs = cs)$$

$$M2b = M0 + s(\Delta MD, bs = cs)$$

$$M3a = M0 + s(MD, bs = cs) + s(WL, bs = cs)$$

$$M3b = M0 + s(\Delta MD, bs = cs) + s(\Delta WL, bs = cs)$$

Drivers of Chowilla–Murray movements

These analyses tested if hydrological conditions affected the probability that a Chowilla-originating movement resulted in individuals entering the River Murray. Movements (per algorithm above) were compared and classified as a Chowilla–Murray movement or not. The full dataset was used and date and associated hydrological conditions at the initiation of all classified movements were extracted. The same GAMMs were applied as above, but response variable was whether movement was classified as a Chowilla–Murray movement or not.

Drivers of long-distance Murray movements

These analyses tested if River Murray discharge affected the probability that a Chowilla–Murray movement would continue for ≥40 km upstream. These movements were typically in upstream rather than downstream direction. As such, all Chowilla–Murray movements that occurred in an upstream direction (e.g. from the Chowilla system through Swifty's Creek and into the River Murray) were extracted. Long distance movements were defined as a fish being detected at the Bank K logging station and/or upstream PIT readers on main channel locks 7–10 within 30 days of initiation of a Chowilla–Murray movement (with no other Chowilla–Murray moves during the 30 days). Given the low number of movements, the dataset was reduced to a single observation per fish (randomly selected) and the random effect was dropped from the models.

Using a General Linear Model (GLM), the response variable was whether a Chowilla–Murray movement continued upstream to Bank K or further. For the base model, only the intercept was included. M1 included cyclic spline for day of year, M2 included River Murray discharge (MD), M2b included ΔMD, and M3 included day of year and MD.

$$M0 = \text{Intercept only}$$

$$M1 = M0 + s(day_{year},\ bs = cc)$$

$$M2a = M0 + s(MD,\ bs = cc)$$

$$M2b = M0 + s(\Delta MD,\ bs = cc)$$

$$M3 = M0 + s(day_{year},\ bs = cc) + (MD,\ bs = cc)$$

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 2017–2023, a total of 328 individuals from five size classes were successfully genotyped (Table 2). This included a total of four larvae and three YOY collected in 2022/23 that resulted from spawning in 2022 and 21 adult fish collected in 2022/23.

Table 2. Number and sizes of Murray cod from Chowilla successfully genotyped across sampling years.

Size Class	2017/18	2018/19	2020/21	2021/22	2022/23	Total
Larvae (10–20 mm TL)	45	58	14	85	**4**	**206**
YOY (20–200 mm TL)	8	3	6	2	**0**	**19**
Juvenile (1 yr) (200–400 mm TL)	-	1	8	1	**3**	**13**
Sub-adult (2–4 yr) (400–600 mm TL)	-	3	20	12	**-**	**35**
Adult (600 mm+ TL)	3	13	5	13	**21**	**55**
Total	**56**	**78**	**53**	**109**	**28**	**328**

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 3). 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 3). Half-siblings, were relatively common among cohorts (53/232) indicating many of the same individuals bred repeatedly over the 5-year study period (Table 4). 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 3. The number (*n*) of full- and half-sibling pairs (dyads) 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 it includes all non-adult life stages as assigned to spawning 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
2022	4	4	0	0	0	0
Total	255	200	55	34	235	232

Table 4. Number of half-sibling pairs across Chowilla that were detected within and between cohort pairs.

Cohort	2017	2018	2019	2020	2021	2022
2017	18					
2018	10	126				
2019	1	3	0			
2020	4	5	0	1		
2021	20	9	0	1	34	
2022	0	0	0	0	0	0

Of the 21 adult Murray cod captured and sequenced in 2022/23 (Table 2), three were assigned as parents of offspring sampled in 2018–2022, increasing the number of known assigned parents sampled to eight (Table 5). This included a 1130 mm adult sampled in 2023 that was the parent of different larvae sampled from the same site in Chowilla Creek in spring 2017 and 2018.

Table 5. Details of parent-offspring pairs for offspring that were assigned with >99% confidence to a candidate breeding adult that was also sequenced.

Offspring ID	Offspring capture details	Assigned Parent ID	Assigned Parent capture details
MC18C3-02	2018 larvae Chowilla Creek	MC_464	2018 adult (inferred age 11; 860 mm), Slaney Creek (lower)
TC_Ch_2022_MC-05	2018 older juvenile (inferred age 3; 396 mm) Bank K	TC_Ch_2022_MC-23	2022 adult (inferred age 22; 1080 mm), Slaney Creek
MC20CH09	2020 larvae Chowilla D/S Pipeclay Creek	MC19CH12	2019 adult (inferred age 16; 985 mm), Slaney
MC21C003	2021 larvae Slaney Creek	TC_Ch_2022_MC-46	2022 adult (inferred age 34; 1200 mm), Pipeclay Creek
MC21C071	2021 larvae Pipeclay Creek	MC19CH11	2022 adult (inferred age 12; 880 mm), Slaney Creek
MC18C1-01	2018 larvae Chowilla Creek	CHOW_MC_11	2023 adult (inferred age 26; 1130mm), Slaney Creek
MC17C1-07	2017 larvae Chowilla Creek	CHOW_MC_11	2023 adult (inferred age 26; 1130mm), Slaney Creek
MC_453	2018 YOY Chowilla - Swiftys Ck	CHOW_MC_30	2023 adult (inferred age 7; 680mm), Chowilla Creek (U/S bridge)
MC_458	2018 YOY Chowilla - Little Slaney	CHOW_MC_30	2023 adult (inferred age 7; 680mm), Chowilla Creek (U/S bridge)
CHOW_MC_23	2023 one year old; 180mm Slaney Creek	CHOW_MC_12	2023 adult (inferred age 15; 960mm), Slaney Creek

Adult contributions to breeding outputs within Chowilla

A total of 268 breeding adults were detected across the study period. Among individual years, the number of breeding adults ranged from 6–86 (Table 6). Overall, most adults (72%) contributed to one sequenced offspring, but 6% of adults were found to contribute to 5 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 and 2022 cohorts to 2.13 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 (*n*) inferred to have contributed to the recruitment of all sequenced offspring across years at Chowilla. *Cohort* is based on the estimated year of spawning. Also presented are % of offspring comprised of larvae compared to other life stages (e.g. YOY), ratio 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	66	68%	83	1.46	1.37	1–6
2018	76	76%	86	1.37	1.47	1–20
2019	11	0%	16	2.00	1.00	1
2020	22	64%	25	1.47	1.36	1–2
2021	90	94%	75	0.94	2.13	1–12
2022	4	100%	6	2.00	1.00	1
All years	269	76%	275	1.21	1.67	1–20

Effective population sizes for Chowilla

Per cohort (including larvae and juveniles) effective population size (N_e) estimates ranged from 49.2–113.9 and 41–128 for the LD and Colony methods, respectively (Table 7). For the 2022 cohort, however, sample size was too small (n = 4) to generate an estimate of N_e. 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). Despite no 2022 cohort estimate of N_e, estimates of effective population size for the adult (5+ years) sample across 2017–2022 (*n* = 55), 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 and a combination larvae and juveniles sampled in subsequent years that were assigned to that spawning year based on the age-length growth curve. *Sample size too small for reliable inference of N_e. ^No sibling pairs were identified within this year meaning Colony was unable to generate an estimate of N_e.

			Larvae & juvenile		**Larvae**	
Cohort	***N***	***N* larvae**	**N_e (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)
2022	4	4	NA*	NA^	NA*	NA^

3.2. Murray cod movement meta-analysis

Hydrology and managed inundations

From October 2007–April 2023, discharge in the lower River Murray (QSA) in the vicinity of Chowilla was highly variable. From October 2007–September 2010, the hydrograph was characterised by low flow, with discharge generally <8000 ML.day^{-1}, before large-scale overbank flooding in spring–summer 2010/11 (peak = 93,000 ML.d^{-1} in February 2011) (Figure 3). Discharge remained relatively high in 2012 (peak ~55,000 ML.d^{-1}) then decreased and remained within channel and predominantly <20,000 ML.d^{-1} from 2013 to early 2016. In October–December 2016, another overbank flood event occurred (QSA peak: ~95,000 ML.d^{-1}; Figure 3) but had a relatively short duration of ~3 months (Figure 4). From 2017–2020, discharge remained within channel, with peak discharges of 12,000–18,000 ML.d^{-1} occurring in October–December. In December 2021, the lower River Murray experienced high within-channel flows (~37,593 ML.d^{-1}) with persistent elevated flows (>20,000 ML.d^{-1}) continuing into early 2022. Hydrology in 2022/23 was characterised by a major prolonged (~six months) flood with a peak discharge of ~185,000 ML.d^{-1} in mid-December (Figure 3).

Operation of the Chowilla Regulator occurred on six occasions over the study period, reaching inundation heights ('Chowilla water level', m AHD) of 19.14 (2014), 17.85 (2015), 19.78 (2016), 18.54 (2018), 19.59 (2021) and 19.55 m AHD (2022) (Figure 3). Operations typically occurred over periods of three–four months (typically August–December). Operations in 2016 and 2022 were immediately followed by natural flooding that inundated the Chowilla floodplain with water levels of ~20 and ~20.6 m AHD, respectively.

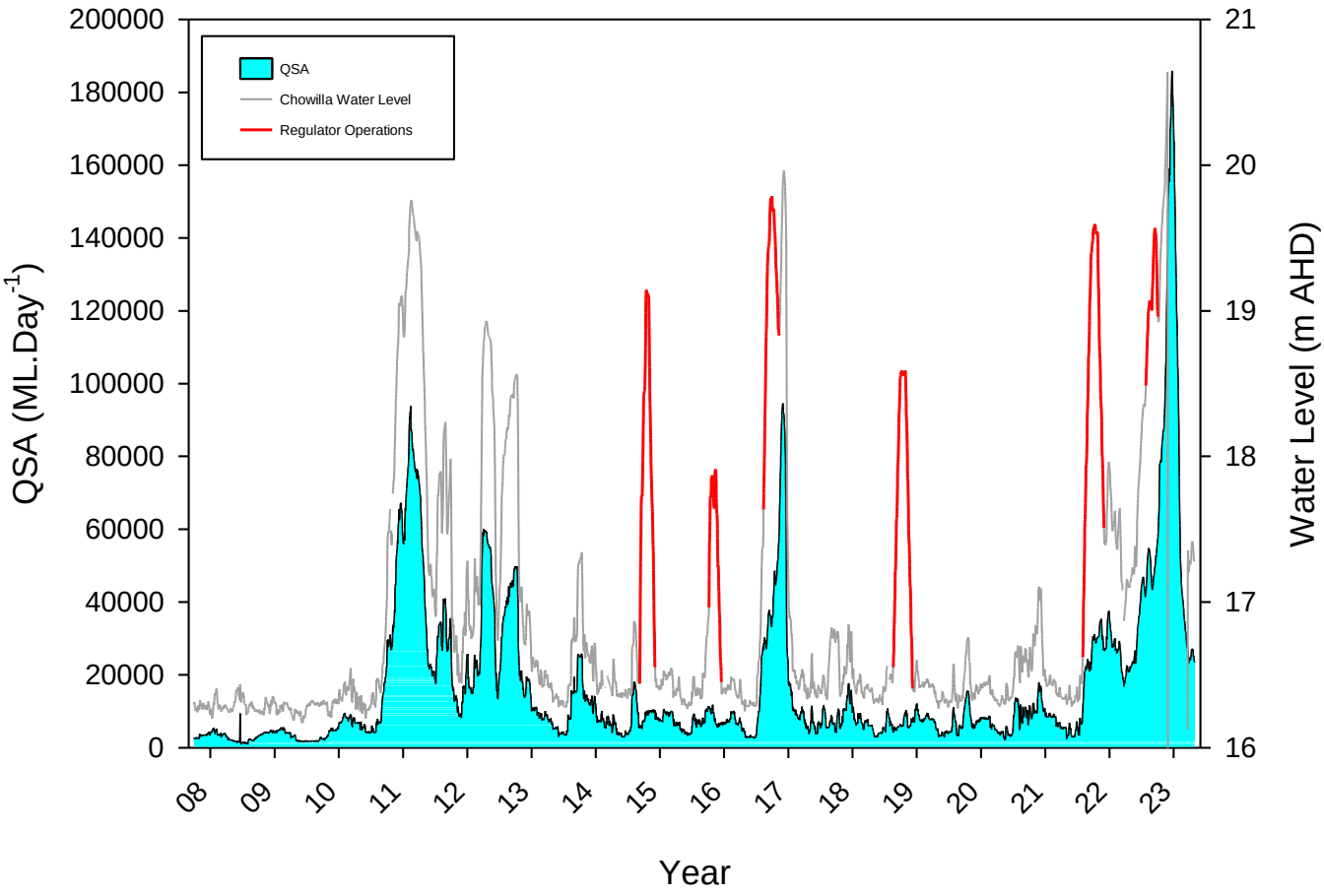

Figure 3. Discharge in the lower River Murray (blue area, QSA, ML.day^{-1}) and water levels within Chowilla (solid grey line, m AHD) from October 2007–April 2023. Period of radio-tracking (black line) reported upon and managed floodplain inundations at Chowilla (red line overlaid on Chowilla Water Level) are also indicated.

General movement patterns

Overall, 102 of 108 radio-tagged Murray cod (~94%) were detected on at least one remote logging station (Figure 4a). Of these detected fish, 61 (56%) were detected at least once in the River Murray, but most ultimately returned to Chowilla, with final detection site for ~81% of individuals being within Chowilla and ~19% in the River Murray. Movements by individual fish varied between years, with high movement activity observed within Chowilla, whilst several fish made upstream movements to as far as Lock 10 on the River Murray (Figure 4b). Individuals were detected for

durations ranging 0–4994 days (Figure 5c), and most (>70%) were detected on four or more receivers (Figure 5b). Overall, most detections occurred from July–October (Figure 5d).

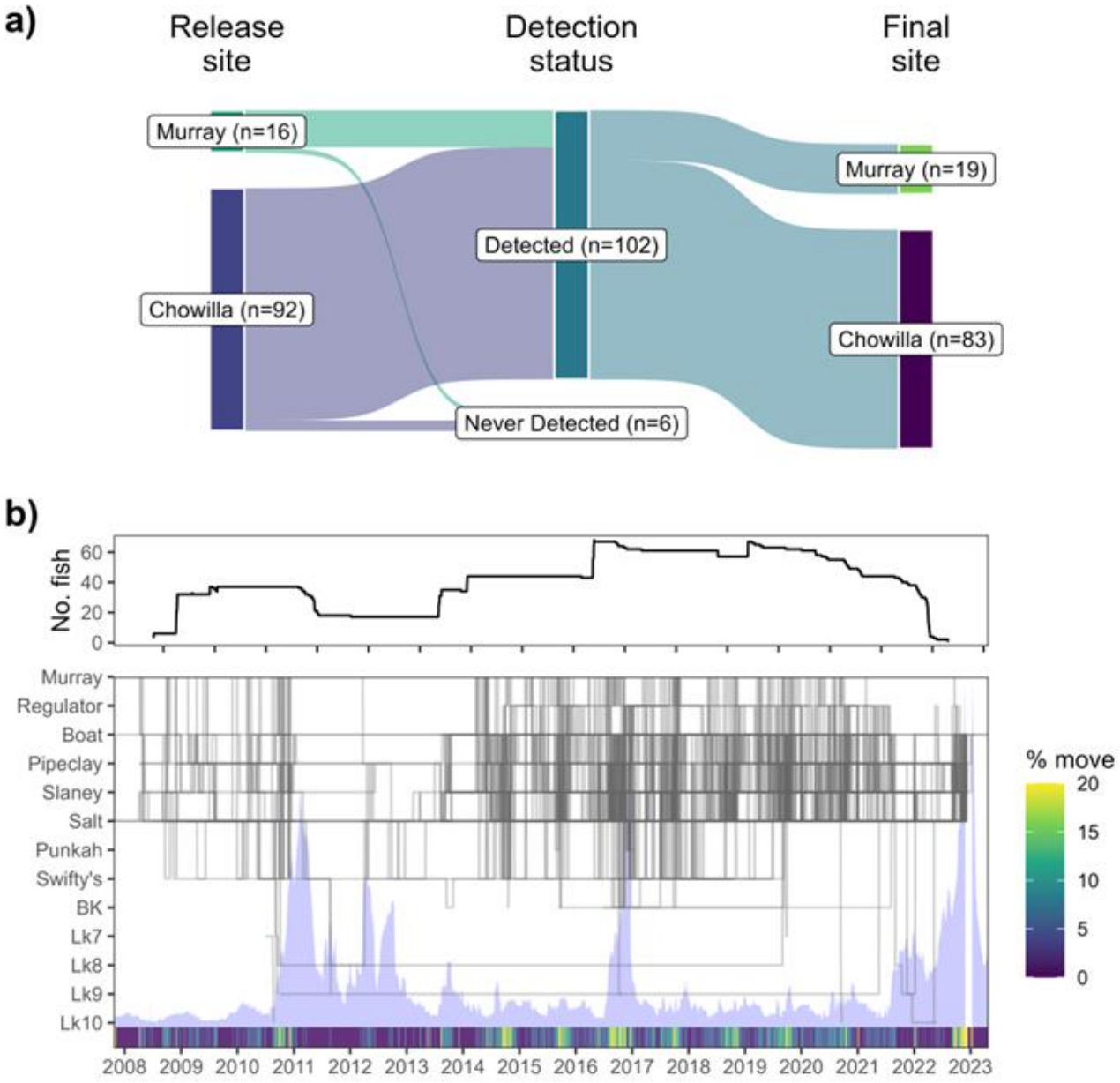

Figure 4. a) Sankey plot depicting capture/release location, detection status ('detected' or 'never detected'), and final location (last known detection within Chowilla or adjacent River Murray), and b) movement profiles for every fish detected on at least one receiver (black lines on bottom plot) and number of tagged fish with active batteries throughout monitoring period (top plot). On bottom plot, blue shaded area represents River Murray discharge (QSA, ML.d^{-1}, see Figure 3 for discharge values) and heatmap at the base of the plot presents the percentage of fish detected 'moving' with the past seven-day period.

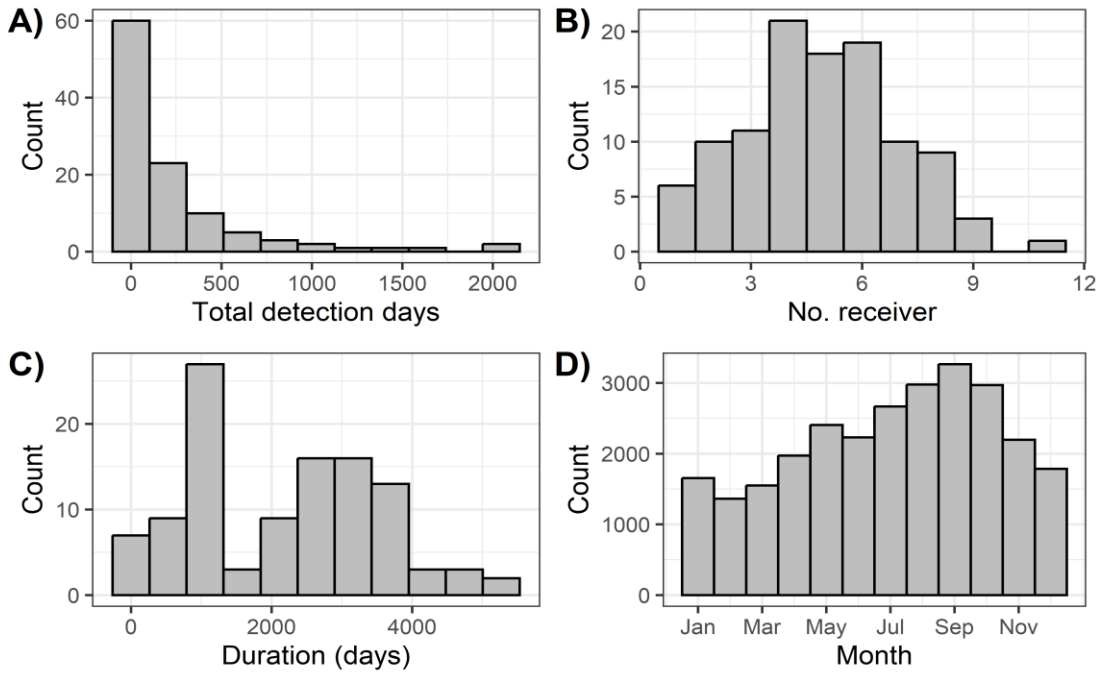

Figure 5. Summary of detection patterns from 2007–2023, depicting a) count of fish against number of days detected, b) count of fish by the number of receivers on which they were detected, c) count of fish against duration of detection (i.e. tag date to last detection) and d) overall number of detections per month.

Of the remote logging stations, the greatest number of individual fish were detected at the junction of Chowilla and Slaney creeks (>80), followed by stations at the junction of Slaney and Salt creeks, and Chowilla and Boat Creeks (60–80) (Figure 6a). Remote logging stations at Punkah Creek and Bank K on the River Murray detected the fewest fish (<20). Estimated linear extent for individual fish across the duration of tracking ranged from <1–220 km, but most individuals (~65%) exhibited linear extents of ≤20 km (Figure 6b).

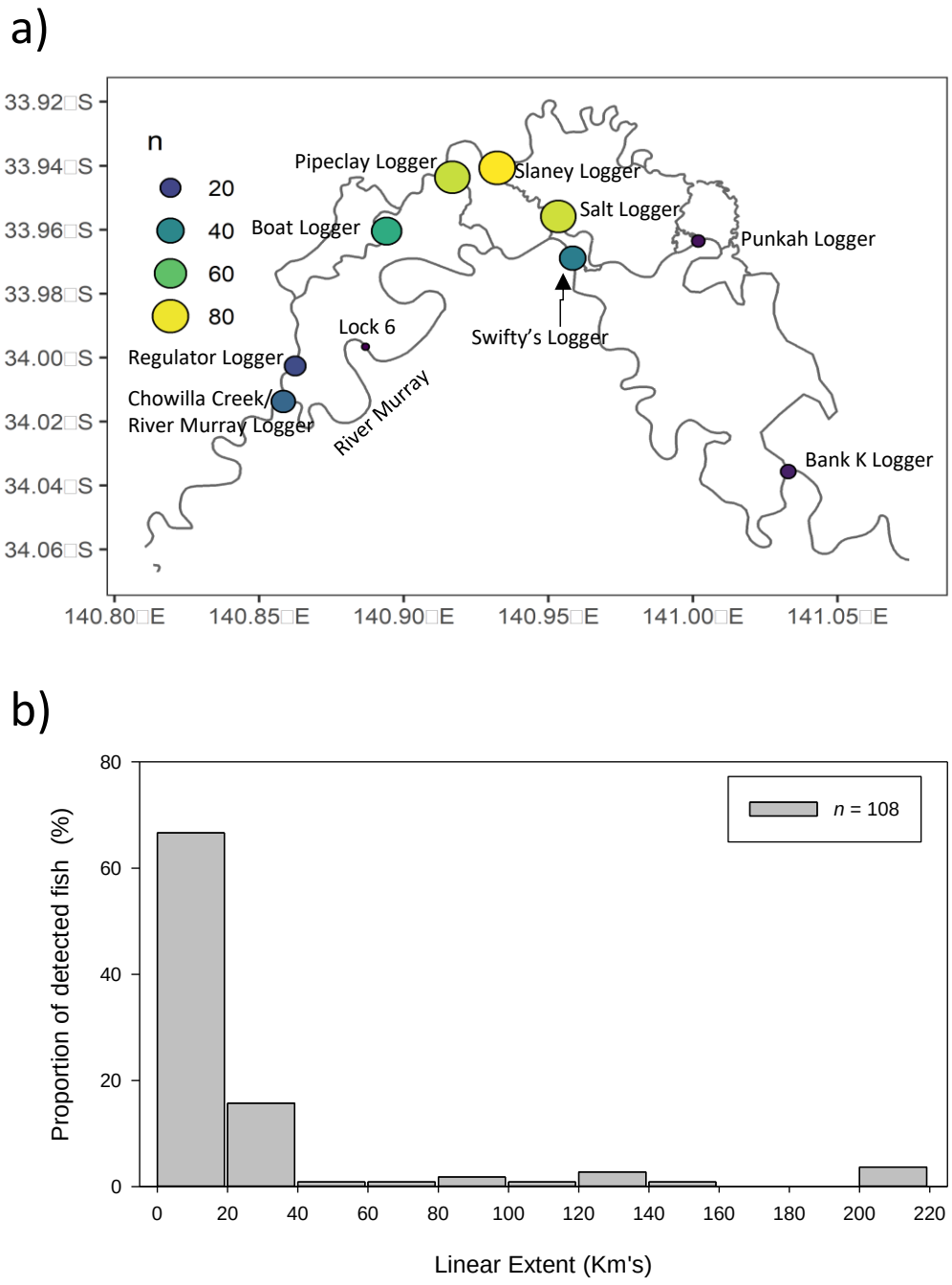

Figure 6. a) Map depicting the number of fish recorded at each remote logging station over the course of the study and b) histogram of estimate linear extent (km) for all tagged fish across the course of the study.

The movement history of the 30 individuals with the longest tracking histories (7–13 years) is plotted in Figure 7 (see Appendix 1 for individual biological data). Some individuals were almost exclusively detected on single receivers across their entire detection duration (e.g. Fish ID 63 on the Salt Logger), while others were regularly recorded on multiple receivers (e.g. Fish ID's 1 and 35) (Figure 7). All fish, except Fish ID 1, exhibited a high level of consistency in intra-annual movement patterns across years. Several individuals exhibited high fidelity to specific locations (loggers) for multiple years (up to 8 years) and then switched to high fidelity for multiple years at other locations; this was notable for fish 9, 12 and 44, which appeared to make distinct and permanent shifts among habitats within Chowilla and the River Murray. Others made intra-annual shifts among habitats within the Chowilla system, that occurred between specific locations during specific seasons repeated across multiple years (e.g., Fish 62 and 73). These most commonly involved movements between Chowilla and Slaney creeks, and within Slaney Creek (i.e., between Slaney and Salt loggers) during winter and spring.

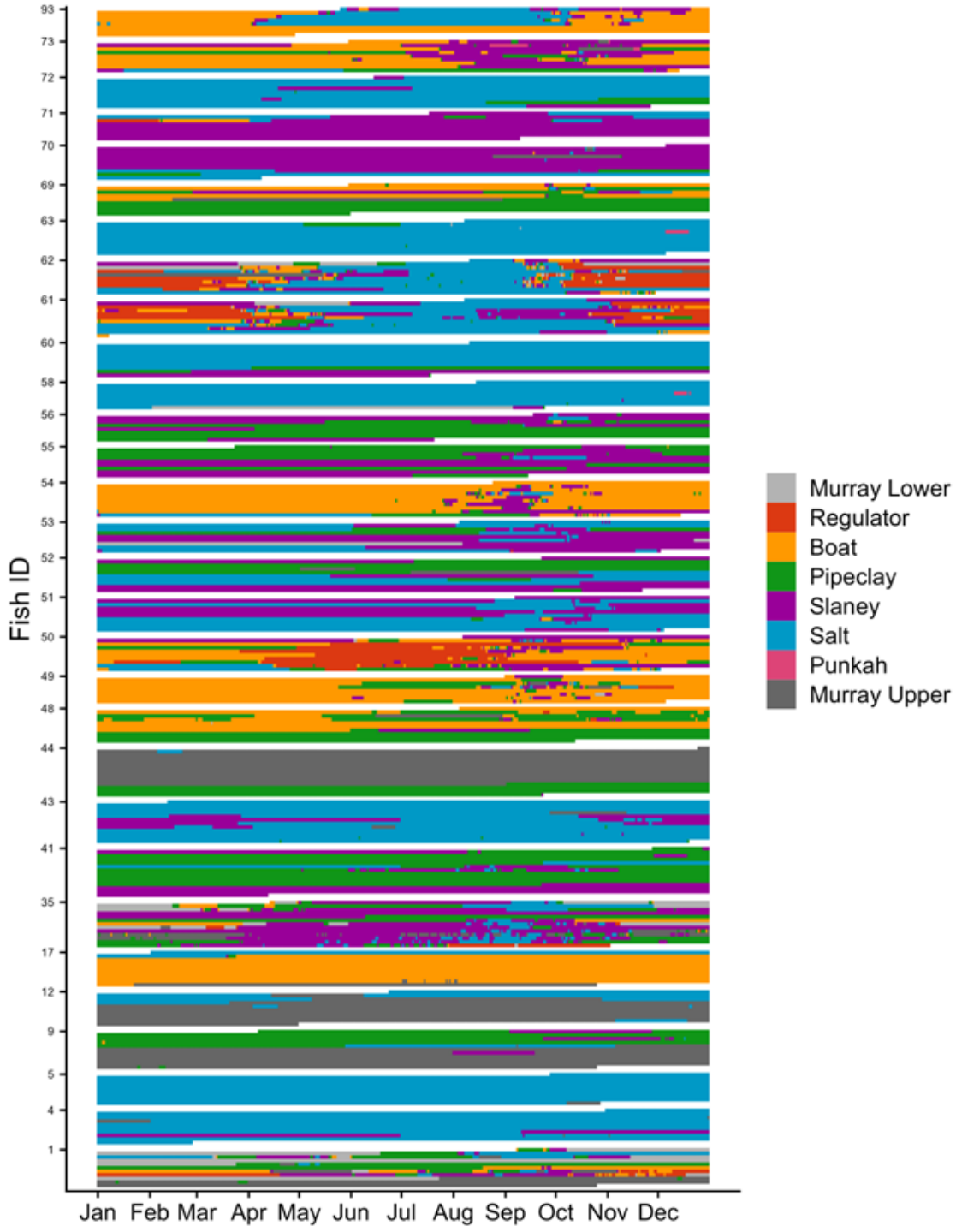

Figure 7. Annual sequences of Murray cod movement presented as daily locations at six locations within the Chowilla Anabranch system (‘loggers) or in the River Murray downstream and upstream of the system. Sequences are presented for the 30 individuals with the longest tracking histories (7–13 years) and multiple years are presented as sequential pixels top to bottom and grouped by Fish ID, and individuals are separated by white rows.

Drivers of movement

For local Chowilla-scale movements, model M3a, which included both Chowilla water level and River Murray discharge, best explained patterns of movement (M0 v M3a: ΔAIC = 73.8). The model that included just River Murray discharge (M2a) had more support than the model with just Chowilla water level included (M1a), but inclusion of both terms improved the model (i.e. M2a v M3a: ΔAIC = 20.5). Further exploration of M3a indicated that all splines affected movement probabilities. Specifically, Murray cod were more likely to move during winter–spring and probability of movement varied across years (Figure 8a-b). After controlling for these effects, we found that both River Murray flow and Chowilla water level affected movement, with the effects of Murray flow larger than Chowilla water levels (Figure 8c–d). Generally, River Murray discharge had a slightly negative effect when <30,000 ML.d^{-1} but an increasingly positive effect at discharge >30,000 ML.d^{-1}, peaking at discharge >60,000 ML.d^{-1}. The effect of Chowilla water levels peaked at ~17.5 m AHD. Models M1a and M2a were then run exclusively for movements that occurred when River Murray discharge was <30,000 ML.d^{-1} and model M1a (i.e. Chowilla water level and splines) became the best fit model.

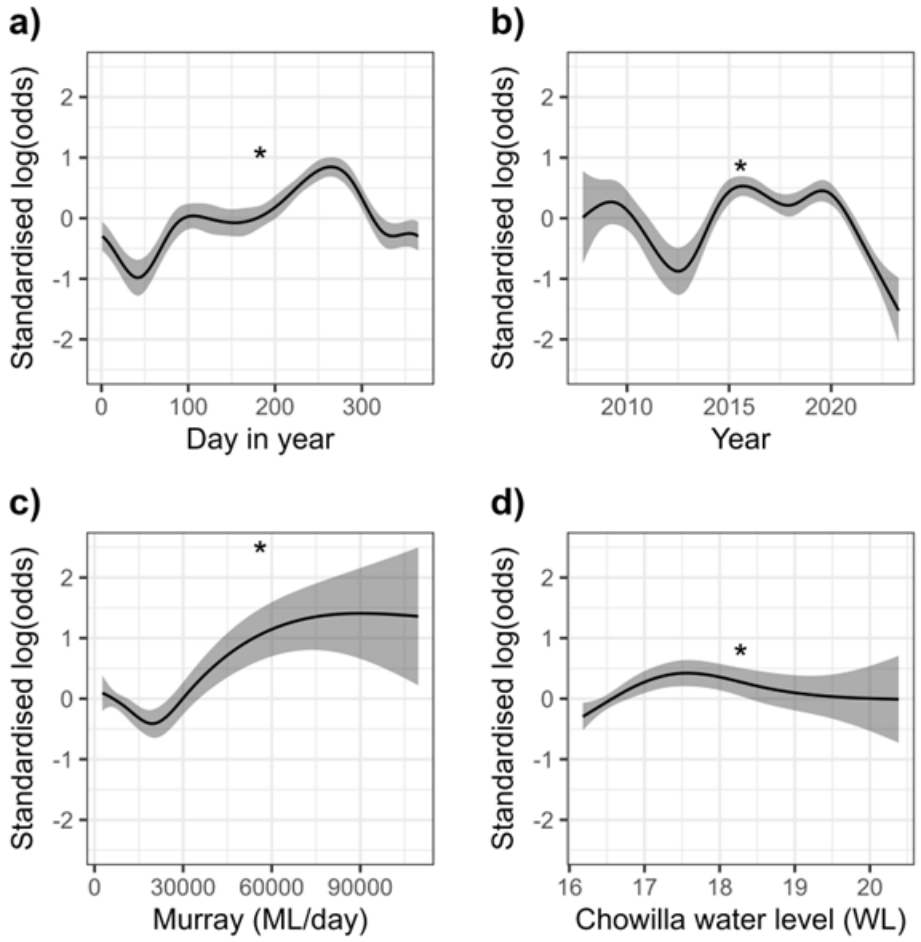

Figure 8. Environmental factors associated with movements of Murray cod at Chowilla. Curves show modelled relationships of probability of movement (with 95% CI, shaded region) in association with a) day of year, b) year, c) River Murray discharge and d) Chowilla water level (m AHD, 'flow Chowilla'). Deviation from 0 is indicative of positive or negative relationships, with ***** indicating a relationship of significance ($p < 0.05$).

With regard to Chowilla–Murray movements, no model was preferred over the base model: e.g. Chowilla water level (M0 v M1a: ΔAIC = -5.3), Murray flow models (M0 v M2a: ΔAIC = 0.1; M0 v M3a: ΔAIC = -5.3) (Figure 9). Murray cod were recorded entering the Murray across different times of year during a large range of Chowilla water levels and River Murray discharges (Figure 10). Within M0, 'year' appeared to have greatest effect on probability of Chowilla–Murray movements, with probability decreasing in later years of the study.

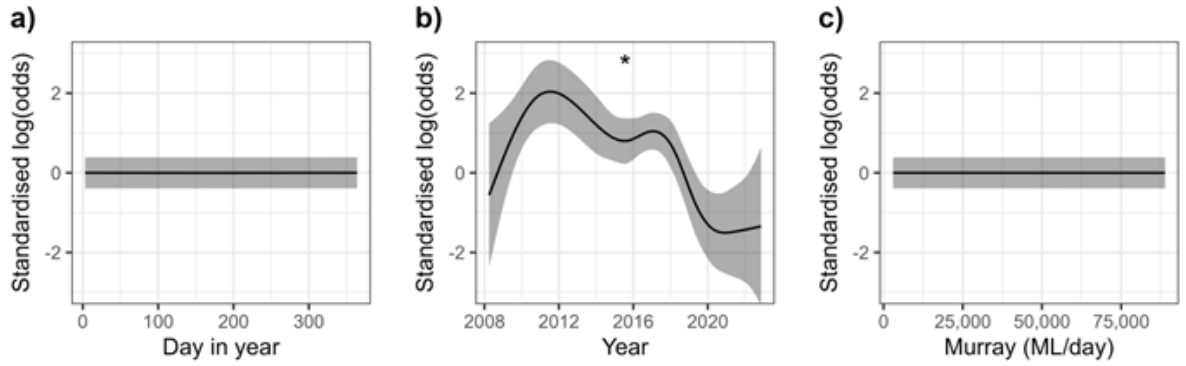

Figure 9. Effect of a) day of year, b) year and c) River Murray discharge (standardised) on odds of a Chowilla–Murray movement. Curves show modelled relationships of probability of movement (with 95% confidence interval (CI), shaded region). ***** indicates significant relationship ($p < 0.05$).

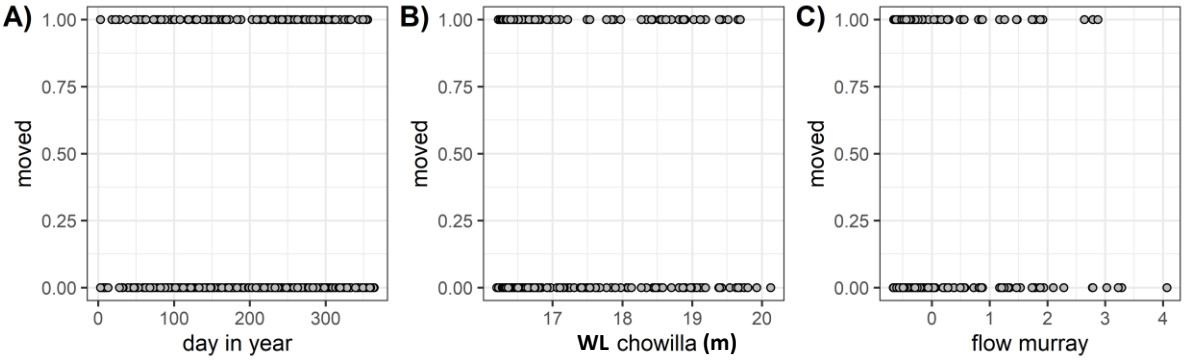

Figure 10. Plot of raw data for Chowilla–Murray movements (1 = Chowilla–Murray movement, 0 = local Chowilla-scale movement) against a) day of year, b) Chowilla flow height and c) Murray flow (standardised).

Regarding long-distance River Murray movements, overall, there were 16 fish that were detected >40 km upstream of Chowilla within 30 days of a Chowilla–Murray movement. Of these 12 reached as far as Lock 7 and one individual was detected at Lock 10. The day of year model, with Murray discharge (M3), was the best fit model (M3 v M0: ΔAIC = 6.9; M3 v M1: ΔAIC = 2.9: Figure 10). Upstream Murray movements were more likely during the end of Winter/early Spring period and during higher Murray flows (Figure 11).

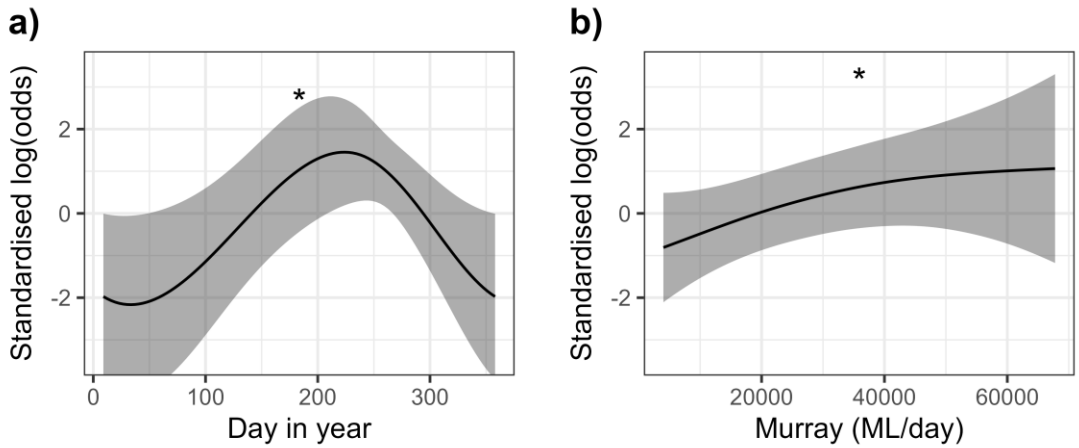

Figure 11. Effect of a) day of year and b) River Murray discharge on the odds of a long-distance upstream movement following a Chowilla–Murray movement. Curves show modelled relationship with 95%CI (shaded region). Ticks on x-axis show the observed data range for that variable. * indicates significant relationship ($p<0.05$).

4. DISCUSSION

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 (the number of offspring contributed by each breeding adult) within the breeding population (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 season) and reproductive skew 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.

In 2022/23, the number of offspring sampled that were sourced from spawning in 2022 were too low to enable estimation of cohort effective population size. Among previous years, 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 non-adult 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.

The cohort effective population size in any given year is typically 6–15 times lower than the overall adult effective population size estimate (Bice et al. 2023a). Per cohort effective population size is likely to underestimate the true number of breeders, due to incomplete sampling of the larval cohort, but may also suggest that not all adults in the population breed in a given year. Per cohort

effective population size estimates can be used to detect signals of population increase or decline over time (e.g. Luikart *et al.* 2020), yet at present there is no conclusive trend at Chowilla. Continued monitoring of the Chowilla Murray cod population will be important to understand the trajectory of effective population size 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 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. Murray cod movement

The results of our meta-analysis highlight the importance of Chowilla for Murray cod in the context of the lower River Murray. Most tagged fish moved <20 km and resided within Chowilla through the duration of their tracking periods. Key areas of residency and distinct movement types, which have previously been identified for this population (Leigh and Zampatti 2013), were evident and associated with specific environmental drivers.

From 2008–2023, the greatest number of radio-tagged Murray cod were detected on remote logging stations adjacent lotic reaches of the Chowilla system, namely the stations at the junctions of Chowilla and Boat, Pipeclay and Slaney creeks, and the junction of Slaney and Salt creeks. This confirms the importance of Slaney and Pipeclay Creeks, and specific reaches of Chowilla Creek, and movements among these, for the Chowilla Murray cod population. Most movements made by individuals occurred among these reaches, yet many fish (>60%) also exhibited movement between Chowilla and the adjacent River Murray, whereby Switfy's Creek was dominant pathway for upstream movements and Chowilla Creek the pathway for downstream movements.

Analysis of the movement histories of the individuals with the longest tracking durations (7–13 years) revealed high levels of inter-annual consistency or repeatability in movement patterns. This includes high levels of site fidelity, but also seasonal movement among specific locations across multiple years. High site fidelity has previously been noted for Murray cod (Leigh and Zampatti 2011, Leigh and Zampatti 2013, Fredberg and Zampatti 2018) but less so for the repeatability in movements. This phenomenon, however, has been observed in several other species of large, long-lived freshwater fishes, including white sturgeon (*Acipenser transmontanus*, Parsley et al. 2008) and walleye (*Sander vitreus*, Elliott et al. 2023). In the case of Murray cod, most of these movements among locations occurred immediately prior and post the spawning season (October–November), suggesting they are related to reproductive activity. The timing of these movements and spawning, as suggested by analyses in this study and others (Leigh and Zampatti 2011, Zampatti et al. 2011, Fredberg and Zampatti 2018), are likely related to circa-annual cues like temperature and photoperiod. Additionally, use of the same locations repeatedly, suggests a level of learning or 'experience' may be involved in choices to return to the same spawning locations (Odling-Smee and Braithwaite 2003, Tibblin et al. 2016). Further investigation of repeatability of migratory patterns in relation to sex, size and environmental conditions is warranted.

Local Chowilla-scale movements were associated with time of year, being most frequent from August–October, and River Murray discharge and Chowilla water levels. Murray cod spawn primarily in October–November (Koehn and Harrington 2006) and elevated movement rates immediately preceding this period are likely related to reproductive behaviour. River Murray discharge (QSA) >30,000 $ML.d^{-1}$ had a positive influence on likelihood of Chowilla-scale movements, with the greatest likelihood at discharges of >60,000 $ML.d^{-1}$. Chowilla water levels also had a positive influence on Chowilla-scale movements, particularly at water levels of ~17.5 m AHD. These water levels can occur during elevated flows and the lower levels of engineered inundations; indeed, when models were run only for River Murray discharge <30,000 $ML.d^{-1}$, Chowilla water levels were a primary determinant of movement. As such, operation of the Chowilla Regulator may influence movement rates in the absence of elevated River Murray discharge.

Contrary to findings for Chowilla-scale movements, our analyses suggested that River Murray discharge and Chowilla water levels were not significant drivers of Chowilla–Murray movements, with movements occurring across a range of flows and water levels. The only significant variable

in the preferred model was ‘year’, which suggested a general trend of decreasing odds of Chowilla–Murray movements over the course of tracking from 2008–2023. Mechanisms behind such patterns are unclear yet may in part be related to the construction of the Chowilla Regulator and operation during spring on several occasions since 2014. On these occasions, concurrent monitoring of radio-tagged Murray cod movement has suggested the downstream and upstream movements of individuals between Chowilla Creek and the River Murray may be impeded by the regulator (SARDI unpublished data). The lack of apparent association of these movements with discharge and water levels was surprising given previous associations suggested for this population (Leigh and Zampatti 2013).

Key drivers of long-distance Murray movements were related to day-of-year in conjunction with River Murray discharge. Indeed, the analyses suggest upstream River Murray movements were more likely to occur during late winter/early spring and during elevated River Murray flow conditions. This result is similar to previous studies that suggest greater propensity for long-distance movements associated with elevated flows and flooding (Leigh and Zampatti 2013).

Overall, like other studies in the MDB, our results suggest that Murray cod life history typically operates over a scale of 10s kms. As such, management at the scale of the Chowilla system and adjacent River Murray is appropriate, noting the interaction among Chowilla and the Lindsay Mullaroo system observed in both the genetic and the movement components of this current project. Continued operation of the Chowilla Regulator and other interventions, notably weir pool manipulation and environmental flow delivery, as well as future effects of climate change, have the potential to influence the hydrology, hydraulics and connectivity of the Chowilla system, and hence, movement and population dynamics of Murray cod. As such, continued investigation of Murray cod movement and habitat use is warranted.

Future studies of Murray cod movement at Chowilla would benefit from an ability to better monitor movement at both fine and larger spatial scales. Acoustic telemetry represents such a technology, and a staged transition from radio- to acoustic-telemetry for ongoing studies of Murray cod movement at Chowilla has commenced. This will allow assessment of creek-scale habitat use in association with management interventions, but will also provide compatibility with technology being used by other researchers (i.e. acoustically tagged Murray cod from Chowilla can be detected on acoustic receivers of other researchers), thus, broadening the effective coverage of movement monitoring. Furthermore, acoustic accelerometer tags are available, which in addition

to movement, can gather data on fish ‘activity’. Use of these tags may provide additional information on Murray cod locomotor behaviour, in association with environmental drivers and system management, that are not evident from monitoring of movement alone.

5. CONCLUSIONS AND RECOMMENDATIONS

In 2022/23, we continued investigations of population dynamics and movement of Murray cod at Chowilla using genetic approaches and an extensive meta-analysis of radio-telemetry data collected from 2008–2023. The results provide important insights on the ecology of Murray cod in the Chowilla region, including:

- Both genetic (high levels of kinship) and movement (limited linear extents) components highlight the generally sedentary nature of Murray cod and importance of managing this population at the scale of the Chowilla and adjacent River Murray system, whilst also considering links to the nearby Lindsay-Mullaroo system.
- Movement meta-analysis highlighted the importance of season, River Murray discharge and water levels within Chowilla on the movement of Murray cod, with movement more likely during August–October, elevated river flow and elevated Chowilla water levels. Regulator operations during August–October have the potential to impede movements between Chowilla Creek and the River Murray. Furthermore, evidence suggests operation of the Chowilla Regulator increases the frequency of local Chowilla-scale movements but subsequent influence on population dynamics is unknown.

For future potential investigations, we recommend the following:

- Continued investigations of Murray cod adult contribution, movement and effective population size using genetic approaches may provide insight on population trends not immediately evident from condition monitoring abundance data. Genetic analyses need not occur annually, but the existing Chowilla Fish Condition Monitoring program provides a means for continued opportunistic collection of genetic samples for later analysis. Interpretive power of this approach will increase with increasing sampling period and sample size.
- Continued investigations of Murray cod movement and habitat use at Chowilla, including strengthening statistical relationships between migratory patterns and environmental conditions, will necessitate tagging more individuals. We recommend the continued tracking of existing radio-tagged individuals while tags have remaining battery, but a transition to acoustic telemetry for newly tagged fish (this has recently commenced in 2024). Furthermore, the use of accelerometer tags, which provide locomotor information in addition to location and time data, may provide greater insight on overall activity.

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7. APPENDIX 1

Table 8. Biological, radio-tag and capture data of 30 individual radio-tagged Murray cod that had the longest tracking histories throughout the study period.

Fish number	Fish freq-code	Date tagged	Capture location	Length (mm)	Weight (g)	Sex	External tag No.	PIT tag No.	Exp Bat Life
1	150.302 (24)	23/10/2007	Upper Slaney Ck	810	9000	N/A	301596	151 489 737	19/11/2016
4	150.302 (23)	24/10/2007	Little Slaney	750	8500	M	301584	151 489 715	20/11/2016
5	150.263 (23)	25/10/2007	Slaney Ck. D/S billabong	630	3500	F	301583	151 489 704	18/11/2010
9	150.483 (24)	1/04/2008	Slaney Ck @ Larval Site	975	19000	M	30635	151 489 424	23/10/2029
11	150.263 (20)	1/04/2008	lower Slaney Ck.	545	2000	F	11603	151 489 426	26/04/2011
17	105.362 (24)	2/04/2008	Slaney Ck. U/S billabong	790	8500	F	30649	151 489 429	30/04/2017
34	150.483 (23)	10/04/2008	Pipeclay Island	1040	20000	F	30627	151 489 391	1/11/2029
41	150.362 (25)	27/11/2008	Pipeclay Island	890	14200	M	300699	151 489 386	25/12/2017
43	150.483 (25)	20/01/2009	Little Slaney Junction	1230	30000	M	300697	145 015 821	13/08/2030
44	150.342 (25)	20/01/2009	Little Slaney	640	4500	M	30921	141 716 526	17/02/2018
48	150.502(50)	15/05/2013	Below Boat logger tower	514	2058	F	307040	982H000361608550	8/06/2016
49	150.362(53)	15/05/2013	US Boat_Chowilla Creek condition monitoring site	825	13000	F	300898	982H000361608531	12/06/2022
50	150.542(52)	16/05/2013	Lower Slaney Creek ~100m us Chowilla junction	910	16500	F	300897	982H000361608564	7/12/2034
51	150.302(52)	16/05/2013	Lower Slaney Creek	955	16200	M	300896	982H000361608602	13/06/2022
52	150.542(55)	16/05/2013	Slaney Creek mid	820	11000	F	300895	982H000361608533	13/06/2022
53	150.521(52)	16/05/2013	Slaney Creek mid	970	22500	M	300894	982H000361608580	7/12/2034
54	150.521(55)	17/05/2013	Chowilla Ck just ds Boat Creek	920	13500	F	300893	982H000361608556	14/06/2022
55	150.502(51)	21/05/2013	Island opposite Pipeclay junction	454	1379	N/A	307038	982H000361608598	14/06/2016
56	150.481(51)	21/05/2013	Slaney about 800m DS Billabong	581	3623	M	307037	982H000361608526	14/06/2016
58	150.481(54)	21/05/2013	Slaney Creek immediately outside billabong	1180	33000	M	300892	982H000361608568	12/12/2034
60	150.502(52)	4/06/2013	Slaney us billabong	1230	38000	N/A	300891	982H000361608544	26/12/2034
61	150.481(53)	4/06/2013	Slaney @ Punkah Island launch	1180	37000	M	300889 + 01319	0000000138891942	26/12/2034
62	150.542(56)	4/06/2013	Slaney @ Punkah Island launch	1170	31000	F	300888	982H000361608566	2/07/2022
63	150.342(53)	4/06/2013	Little Slaney	1100	29000	M	300887	982H000361608539	2/07/2022
69	150.481(50)	5/12/2013	Chowilla Ck 400m u/s Boat Ck Junction	583	3400	N/A	307032	982H000361608554	30/12/2015
70	150.521(59)	5/12/2013	Slaney/Chowilla Creek Junction (big snag)	770	10000	M	300853	982H000361608542	2/01/2023
71	150.342(51)	5/12/2013	Slaney Creek ~ 1500m u/s Chowilla	450	1300	N/A	307031	982H000361608552	29/12/2016
72	150.481(52)	5/12/2013	Slaney Creek (big snag d/s old island)	1190	32000	M	300158	982H000199037333	28/06/2035
73	150.542(53)	6/12/2013	Chowilla Ck u/s boat	1010	20000	F	300854	982H000361608609	29/06/2035
93	150.542(54)	24/05/2016	Slaney~200m DS Salt Junction	950	16500	F	300213	982H000361607054	21/06/2025

8. APPENDIX 2

Table 9. GAMM statistical tables for the best model.

Analysis	Type	Term	Estimate	Stat	P
Q1: Chowilla	fixed	Intercept	-3.493	-30.717	<0.001
		wk_prev	1.563	21.621	<0.001
			EDF	Stat	P
	spline	day_year	7.1	200.8	<0.001
		date	7.5	189.9	<0.001
		MF	3.5	53.8	<0.001
		WL	2.8	20.5	<0.001
		fish_id	80.7	812.5	<0.001
Q2: To Murray	fixed	Intercept	-2.71	-10.544	<0.001
			EDF	Stat	P
	spline	day_year	0	0	0.39
		date	5.2	74.7	<0.001
		MF	0	0	0.92
		fish_id	40.3	89.8	<0.001
Q3: Up Murray	fixed	Intercept	-0.823	-2.18	0.029
			EDF	Stat	P
	spline	day_year	1.6	6.3	0.014
		MF	1.1	3	0.05

9. APPENDIX 3

Table 10. Model selection results showing AIC results. Note - dWL = ΔWL; dMF = ΔMF

Analysis	Model	Rank	dAIC	AIC
Q1: Chowilla	M3a: s(day_year) + s(date) + s(MF) + s(WL) + (1|fish_id) + moved_prev	1	0.0	7,887.5
	M2a: s(day_year) + s(date) + s(MF) + (1|fish_id) + moved_prev	2	20.5	7,908.0
	M2b: s(day_year) + s(date) + s(dMF) + (1|fish_id) + moved_prev	3	51.6	7,939.0
	M3b: s(day_year) + s(date) + s(dMF) + s(dWL) + (1|fish_id) + moved_prev	4	53.6	7,941.1
	M1a: s(day_year) + s(date) + s(WL) + (1|fish_id) + moved_prev	5	54.1	7,941.5
	M0: s(day_year) + s(date) + (1|fish_id) + moved_prev	6	73.8	7,961.3
	M1b: s(day_year) + s(date) + s(dWL) + (1|fish_id) + moved_prev	7	74.7	7,962.2
Q2: To Murray	M1b: s(day_year) + s(date) + s(dWL) + (1|fish_id)	1	0.0	441.7
	M3b: s(day_year) + s(date) + s(dMF) + s(dWL) + (1|fish_id)	2	0.1	441.8
	M2b: s(day_year) + s(date) + s(dMF) + (1|fish_id)	3	0.1	441.8
	M2a: s(day_year) + s(date) + s(MF) + (1|fish_id)	4	0.1	441.9
	M0: s(day_year) + s(date) + (1|fish_id)	5	0.2	442.0
	M1a: s(day_year) + s(date) + s(WL) + (1|fish_id)	6	5.6	447.3
	M3a: s(day_year) + s(date) + s(MF) + s(WL) + (1|fish_id)	7	5.6	447.3

Analysis	Model	Rank	dAIC	AIC
Q3: Up Murray	M3: s(day_year) + s(MF)	1	0.0	53.7
	M1: s(day_year)	2	2.9	56.6
	M2a: s(MF)	3	4.2	58.0
	M0: 1	4	6.9	60.6
	M2b: s(dMF)	5	6.9	60.6