Inland Waters & Catchment Ecology

Chowilla Icon Site Intervention Monitoring 2020/21

C. M. Bice, J. O'Dwyer, J. F. Fredberg, K. Harrison and B. P. Zampatti

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January 2023

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 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 in the Chowilla system, an *intervention monitoring* program supports targeted investigations to inform adaptive management.

Intervention Monitoring in 2020/21 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 2020 and, together with historic samples, undertake genetic analyses using kinship approaches to estimate reproductive variance, movement and breeding population size;
2) Implement a fine-scale acoustic receiver array in Boat Creek and acoustically tag large-bodied fishes to support a subsequent investigation of habitat use, movement and fish passage in Boat Creek; and

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

Murray cod reproductive variance, movement and effective population size

Samples of tissue from larval, juvenile, sub-adult and adult Murray cod collected from Chowilla from 2017–2021, together with samples of juvenile, sub-adult and adult Murray cod collected from the nearby Lindsay-Mullaroo system, Victoria, underwent 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 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 164 offspring sequenced from 2017–2021 were estimated to be derived from 216 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 216 adults. 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. Kinship relationships between samples from Chowilla and Lindsay-Mullaroo provided evidence of movement and a moderate degree of demographic connectivity between these two systems. A limited sampling period and low sample numbers means the results of these analyses must be interpreted with caution. Yet, interpretive power will increase with collection and sequencing of further samples and this genetic approach is promising for providing greater insight on the potential effects of flow, structure operation and connectivity on the population dynamics of Murray cod.

Fish movement and habitat use in Boat Creek

This component involved the initiation of an investigation of large-bodied fish movement and passage in Boat Creek to inform future management of connectivity and flow in this creek. In 2020/21, we established an array of 26 acoustic receivers in Boat Creek and the broader Chowilla system with the aims of: 1) providing fine-scale tracking of fish movement within Boat Creek; 2)

assessing passage and delay at the Boat Creek bridge and associated rock bank; and 3) assessing broader-scale movements among major tributaries within the Chowilla Anabranch and the River Murray. In Boat Creek, we captured and implanted 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) with acoustic transmitters and their movements will be monitored across 2021/22.

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

The spatial ecology of Murray cod in the Chowilla system and 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 telemeted logging stations (ATS radio receiver/loggers) that have remotely monitored the movement of >100 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, software upgrades were installed in all logging towers, while extensive upgrades, including solar panels and regulators, and battery replacement, were undertaken at two loggers. In 20/21, a total of 47 tagged Murray cod had active tags and were detected within or adjacent the Chowilla system via manual tracking or on remote logging stations. Based on estimated battery life, by end-2022 the tagged Murray cod population will be reduced to approximately 30 fish. Of these, most tags are due to expire in 2026 and beyond. As such, the continued use of radiotelemetry to study spatial ecology of Murray cod at Chowilla and the adjacent River Murray will be reliant on tagging further individuals during 2022–2024.

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). 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 salinisation 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 earnest in 2014, with the construction of the Chowilla Creek Regulator and ancillary structures, which enable large-scale engineered floodplain inundation with the aim of improving ‘ecological condition’ of the floodplain.

Operation of the Chowilla Regulator, whilst potentially promoting localised ecological benefits for floodplain biota, also poses ecological risks, particularly for aquatic biota that depended 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 et al. 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 in the Chowilla system, an *intervention monitoring* program supports targeted investigations to inform adaptive management. The current report presents the fish intervention monitoring program for 2020/21, which included three specific but related components. The background for each is provided below.

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

Understanding population dynamics is critical for management and conservation. While knowledge of trends in relative abundance and demography are important, other reproductive characteristics, including the relative contribution of different breeding adults is also critical. Specifically, for long-lived species, such insights may provide clarity on the impact of specific threats (e.g. regulator operation and altered hydrodynamics) 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 long-term pair bonding and intra-season polygamy, as well as estimation of the number of successful breeding adults and reproductive variance (disproportionate contribution from individual breeders) in a population across time. These parameters were estimated for Murray cod in the Chowilla anabranch using larvae that were collected from the 2017 and 2018 spawning seasons (Gibbs et al. 2020, O'Dwyer 2022), and the techniques appear a viable approach to complement existing TLM Condition Monitoring and provide greater insight on the influence of system management on Murray cod reproduction and population dynamics in the Chowilla region. Furthermore, allied analyses of samples from the nearby Lindsay-Mullaroo anabranch system, Victoria, could provide insight on interaction between these two critical sites for Murray cod in the lower River Murray.

Fish movement and habitat use in Boat Creek

Boat Creek is one of several influent creeks to 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), that creates a channel constriction that may present a barrier to upstream 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 flow 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 (2009), however, generally low flow in the lower River Murray prompted the placement of rock 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 movement at the Boat Creek Bridge has been considered and a fishway scoping and design report developed. Nonetheless, aspects of the use of lower Boat Creek by large-bodied fishes remain unknown. This includes: the abundance of various species; nature of habitat use (resident, seasonal or vagrant); and the influence of the bridge on movement. Acoustic telemetry provides a means of investigating the movement of large-bodied fish within Boat Creek, which will inform management of creek hydrology (e.g. rock removal) and the potential need for a fishway.

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

The movement of Murray cod in the Chowilla system and 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 telemeted 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 at 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, antennas, etc.) 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 in the region, several new components and software upgrades need to be applied. This will likely occur over time in a staggered approach, with two stations upgraded in 2020/21.

In addition to infrastructure upgrades, studies of movement are reliant on maintaining a population of tagged fish. In 2020/21, the status of tagged Murray cod in the Chowilla Anabranch 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 and requirements for increasing the tagged fish sample size for future studies.

1.2. Objectives

The primary objectives of this study were to:

1) Capture Murray cod larvae and young-of-year (YOY) derived from spawning in 2020 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) Implement a fine-scale acoustic receiver array in Boat Creek, and acoustically tag large-bodied fishes to support a subsequent investigation of habitat use, movement and fish passage in Boat Creek; and
3) Assess status of the radio-tagged Murray cod population and radio-telemetry infrastructure and purchase equipment for upgrade of two 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).

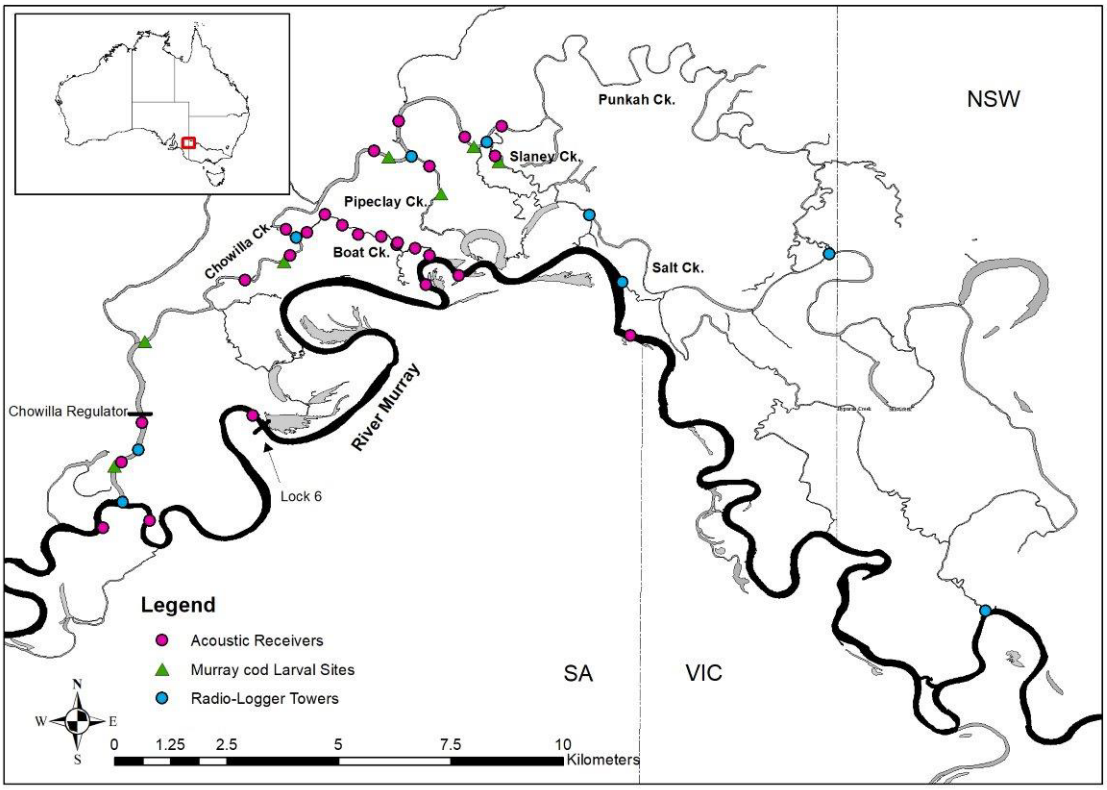

Figure 1. Map of the Chowilla Anabranch System on the River Murray, South Australia. The purple circles indicate the location of the 26 acoustic receivers deployed throughout the system to obtain movement data from acoustically tagged fish in Boat Creek (n = 38). The blue circles indicate the location of the 9 fixed radio telemetry stations (logger towers) used to track tagged Murray cod within the Chowilla Anabranch and the green triangles represent the 7 sites associated with the collection of Murray cod larvae to undergo genetic analyses to provide an estimate of breeding population size and reproductive variance within Chowilla.

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

Sampling

Investigation of Murray cod kinship, reproductive variance, migration and effective population size used tissue collected as part of the current project and existing collections. From Chowilla, existing collections of larvae were available from spring 2017 and 2018 (see Gibbs et al. 2020), while in 2020/21, sampling of Murray cod derived from spawning in spring 2020 occurred via two approaches: 1) larval sampling in spring 2020; and 2) sampling of YOY in autumn 2021 during annual condition monitoring electrofishing surveys (see Fredberg et al. 2021). In addition, to supplement larvae and YOY, tissue samples of larger/older juvenile, sub-adult and adult Murray cod opportunistically collected in autumn 2021 and preceding years (2019 and 2020) were also analysed. Analysis of samples from large fish potentially allows 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 2018–2021 were also included to investigate movement of fish between Chowilla and Lindsay-Mullaroo.

In 2017, 2018 and 2020, 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 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 in the Chowilla Anabranch in 2017, 2018 and 2020, including the number of sampling events for drift nets and light traps.

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

YOY, juvenile and sub-adult/adult Murray cod samples were collected during annual Chowilla fish condition monitoring in autumn of 2017–2021 using a vessel mounted 5 kW Smith Root Model GPP electrofishing system (Fredberg et al. 2021). Samples from the Lindsay-Mullaroo were collected in autumn of 2018–2021 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.

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.

After DArT analytical pipelines, all loci were analysed for Hardy–Weinberg equilibrium (HWE), and for non-neutrality using the dartR v 1.9.9.1 package in R v 4.1.0 (R Core Team 2013, Gruber et al. 2018). All markers were found to be in HWE and no evidence of non-neutrality was observed. SNPs were further filtered using the dartR package (Table 2). All SNPs were filtered at a reproducibility value of 0.98, retaining SNPs that were consistent in at least 98% of technical replicates sequenced as part of the Dartseq process. The data were filtered to remove any SNP that was missing in >30% of individuals. All individuals were then filtered for a maximum allowable amount of missing data of 30%. All SNPs were then filtered to remove alleles that were present in <2% of all individuals and only a single SNP per sequence tag was retained (removal of secondary SNP tags per sequence).

Kinship analysis and assignments using Colony

Sibling relationships were identified using the maximum likelihood-based program Colony v2.0.6.5 (Jones and Wang 2010). Colony was run under the settings of combined pairwise and full-likelihood score, a genotyping error rate of 1%, updating allele frequencies, assumed inbreeding, polygamy for both males and females, and five replicates. Colony was run with adults listed as putative adults and all samples (including adults) listed as siblings, and with subsets of individuals to equalise yearly sampling. Colony requires all putative parents to be listed with known sex. As most adults were of unknown sex, they were assigned as the same sex (male) during analysis. The run with all adults as siblings was undertaken to confirm that no unknown sexed Murray cod adults failed to be assigned to an offspring due to being listed as the wrong

sex (if adults were classed as siblings of an offspring, the Colony run with adults listed as putative adults was re-run with that adult in the opposite sex column).

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

The Colony output file containing the predicted adults who contributed to each offspring sequenced (BestCluster file) was imported into R and data was filtered to only include relationships identified at >99% confidence within Colony. Using sorting, filtering, and counting functions within the packages *plyr, dplyr, base* and *stats*, the number of parents contributing to offspring in each year's samples was calculated. Yearly counts were combined and the cumulative contribution of each parent across all years was calculated to measure the reproductive variance among adults detected within this study.

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 was imported into R. 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 (N_e) of the Chowilla Murray cod population was calculated using two methods. First, the linkage disequilibrium method implemented in the program NeEstimator v2 was used (Do et al. 2014). N_e estimates were calculated using a PCrit cut off value of 0.01, and 95% confidence intervals were generated using a parametric (χ2-based method) of cross loci comparisons. Second, the kinship estimation method implemented in Colony was used with identical Colony run parameters as described above in '*Kinship analysis and assignments using Colony'* (Wang 2009). N_e estimates were calculated for all individuals together, and for individual yearly subsets based on the estimated birth year of each sample as inferred from the estimated age of each Murray cod based on the length-age relationship of Todd and Koehn (2009). For the whole Chowilla population calculations, N_e estimates were additionally calculated with the presence, and the absence of breeding adults included in the population.

2.3. Fish movement and habitat use in Boat Creek

Acoustic receiver array

In June 2021, an array of 26 VEMCO VR2W acoustic receivers was deployed in the Chowilla system (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; and 3) assessment of broader-scale movements among major tributaries within the Chowilla Anabranch and the River Murray. 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).

Fish Capture and Surgery

Across two events, 5–9 July and 13–16 December 2021, fish were captured from Boat Creek 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 (*Cyprinus carpio*), 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.

2.4. 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 the Chowilla Anabranch (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 80W solar panel. As a fish passes within ~600m 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 week 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, tower diagnostics were assessed, and sites visited in July 2021. Several meetings were conducted with Karltek to discuss software and hardware upgrade needs.

Status of tagged fish population

To determine the status of tagged Murray cod still active within the Chowilla Anabranch system a combination of remote and manual tracking was undertaken. Manual tracking occurred in May 2021, whereby core Murray cod habitat within the Chowilla Anabranch (Slaney Creek, Chowilla Creek, Pipeclay Creek, Bank K, Swifty's Creek, Salt Creek and the Murray River main channel adjacent to Chowilla Creek) was 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 Chowilla Anabranch 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 and effective population size

Sampling and sequencing

From sampling conducted from 2017–2021, tissue samples were obtained from 256 individual Murray cod from five size classes (Table 2). This includes a total of 14 larvae and nine YOY collected in 2020/21 and derived from spawning in 2020. In addition, from 2019-2021, 101 samples were collected from fish ranging 200–1180 m TL. Of the 256 samples, 194 yielded DNA of sufficient quality for further analysis.

Table 2. Number and size class of Murray cod sampled across years from the Chowilla Anabranch and used for genetic analysis to determine the number of successful breeding adults and reproductive variance at Chowilla through time. Numbers of individuals that yielded quality DNA samples are included in brackets.

Size Class	2017	2018	2019	2020	2021	Total
Larvae (10 – 20 mm TL)	52 (44)	67 (56)	-	14 (13)	-	**133 (113)**
YOY (20 – 200 mm TL)	-	8 (8)	3 (3)	-	9 (9)	**20 (20)**
Juvenile (200 – 400 mm TL)	-	-	3 (2)	-	25 (21)	**28 (23)**
Sub-adult (400 – 600 mm TL)	-	-	6 (4)	-	13 (9)	**19 (16)**
Adult (600 mm+ TL)	-	2	30 (14)	-	24 (6)	**54 (22)**
Total	**52 (44)**	**77 (69)**	**42 (23)**	**14 (13)**	**70 (45)**	**256 (194)**

Adult contributions to breeding outputs within Chowilla

Of the samples that yielded DNA of sufficient quality, a total of 164 individuals were assigned as juveniles from spawning that occurred during spring from 2017–2021. This resulted in an estimated total of 216 adults that were found to contribute to an average of 1.52 offspring per adult (Table 3). Overall, most adults (~80%) contributed to one sequenced offspring (Figure 2). Approximately 5% of adults, however, were found to contribute to 5 or more offspring across years, and one adult contributed to 20 offspring (Figure 2).

Table 3. Numbers of breeding adults inferred to have contributed to the recruitment of all sequenced offspring in Chowilla

Year of sampling	Offspring sequenced	Adults inferred	Ratio of breeding adults inferred to offspring	Mean offspring each adult contributed towards
2017	44	55	1:1.25	1.6
2018	67	83	1:1.24	1.61
2019	7	14	1:2	1
2020	13	19	1:1.46	1.37
2021	33	64	1:1.94	1.03
Total	164	216	1:1.32	1.52

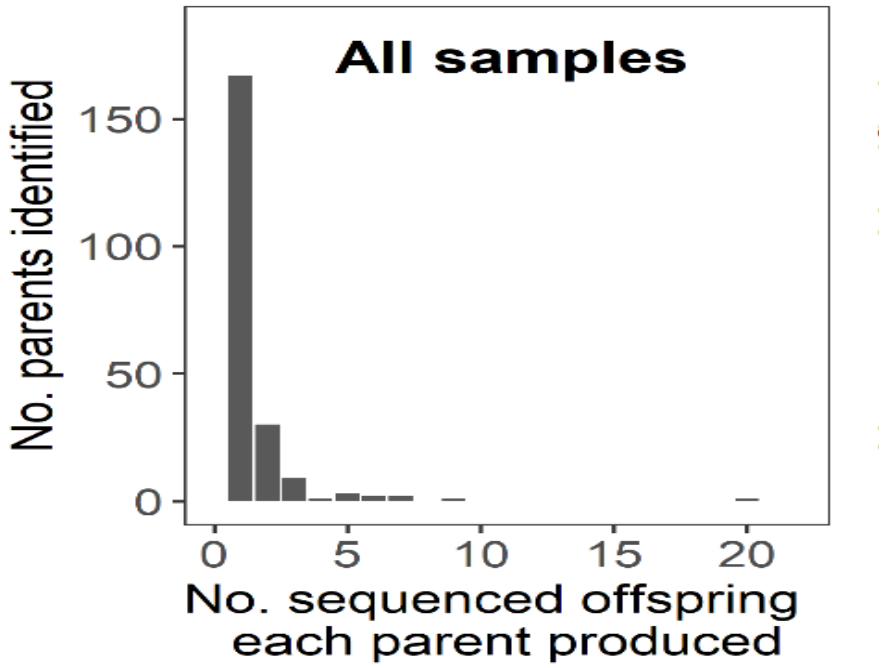

Figure 2. Plots of parental contribution and number of offspring each parent contributed towards across 2017-2021.

The average number of offspring each adult contributed towards varied between years ranging from 1 (every offspring had a unique pair of adults) in 2019 to 1.61 (each adult on average had 1.61 sequenced offspring within this study) in 2018 (Table 3). 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). More adults were also detected in years with higher

sampling intensity for all years except for 2021 and 2017 where more adults were inferred in 2021 despite having 11 fewer offspring sequenced (Table 3).

Kinship and movement among Chowilla and Lindsay-Mullaroo

A total of 304 kin dyads (full-sibling and half-sibling pairs) were detected from Chowilla representing 114 individuals (offspring). 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).

Of offspring sequenced, four offspring were assigned to adults that had been captured, tissue taken, and DNA sequenced. Three distinct adults were assigned to those four offspring, one contributing to two offspring, and the other adults one offspring each. All three adults were of unknown sex but were large (>800 mm TL) and estimated to be at least 10 years old.

In addition to the 114 offspring representing half- and full-siblings from Chowilla, a further 35 were sequenced from the Lindsay-Mullaroo system, and collectively identified 340 kin dyads. In total, 33 individuals exhibited kinship relationships indicative of movement events between Chowilla and Lindsay-Mullaroo. This could have occurred as either a reproductively mature adult migrating and spawning in both systems or one of a sibling pair moving between systems. As all results are generated between kin pairs (or larger families), the 33 individuals were indicative of sixteen movement events between Chowilla and Lindsay-Mullaroo.

Effective population sizes for Chowilla

Estimates of N_e varied between the NeEstimator and Colony methods, with Colony producing a broader range of estimates. Nonetheless, 95% confidence intervals of the two methods overlapped for each specific estimate (yearly and total), adding confidence to the reported ranges of N_e (Table 4–5). In 2019, due to low sample sizes and absence of kin pairs (full-siblings or half-siblings), no N_e estimates could be calculated. For the remaining years, N_e estimates from both Colony and NeEstimator were lowest for 2018, despite this year having the largest cohort size, suggesting either substantially fewer breeding adults or adults with lower genetic diversity (inbred) contributing to offspring. In contrast, similar effective population sizes were estimated for 2017

and 2020, suggesting both spawning seasons had a similarly diverse cohort of breeding adults that contributed to the sampled offspring (Table 4). Population-wide estimates of N_e across 2017–2020 were higher than any individual breeding season indicating that not all breeding adults were detected in each breeding season.

Table 4. Effective population sizes of Murray cod from Chowilla broken down into yearly subsets (based on year of birth estimated based on the Murray cod age-length growth curve), without adults included. *Sample sizes were found to be too small for reliable a reliable inference of N_e. † No sibling pairs was identified within this year, meaning Colony is unable to generate an estimate of N_e. Values in brackets represent 95% confidence intervals for each estimate.

Year of birth	Cohort sample size	NeEstimator (just offspring)	Colony (just offspring)
2017	64	89.1 (87.6–90.6)	116 (81–165)
2018	69	51.5 (51.0–52.1)	35 (23–56)
2019	10	NA*	NA†
2020	20	86.1 (81.6–91.1)	109 (56–476)

Table 5. Total effective population sizes of Murray cod from Chowilla with the presence and absence of adults included in the estimation. Values in brackets represent 95% confidence intervals for each estimate.

	Sample size	NeEstimator	Colony
With adults	194	174.9 (172.9-176.9)	148 (115-187)
Without adults	171	146.7 (145.1-148.3)	148 (116-189)

3.2. Fish movement and habitat use in Boat Creek

From 5–9 July and 13–16 December 2021, 38 large-bodied fish were captured from Boat Creek (downstream and upstream of the bridge) and implanted with acoustic tags (Table 6). Tagged fish comprised four native species, namely Murray cod (*n* = 7; 360–470 mm TL; 639–1425 g), golden perch (*Macquaria ambigua*: *n* = 17; 364–494 mm TL; 745–2000 g), freshwater catfish (*n* = 4; 318–580 mm TL; 257–1500 g) and silver perch (*Bidyanus bidyanus*: *n* = 1; 382 mm FL; 782 g), and non-native common carp (*n* = 9; 318–685 mm TL; 592–4692 g) (Table 6).

Table 6. 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 6 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

3.3. Murray cod radio-telemetry

3.3.1. Infrastructure maintenance

Of the nine remote logging stations at Chowilla, five were offline or operating intermittently due to issues with power supply caused by battery degradation and insufficient solar energy input. Furthermore, all units required software upgrades to KLK3000 units. In addition, current modems and antenna for transmitting data are 3G technology, which will soon be obsolete (need to upgrade to 4G or 5G).

In August 2021, KLK3000 units were removed from each logger tower and sent to Karltek for diagnostics and software upgrades. Two specific logging stations: 1) at the junction of Slaney and Chowilla creeks; and 2) at the junction of Chowilla Creek and the River Murray, were fully upgraded. This included: 1) replacement of existing 80W solar panels with 170W panels; 2) replacement of original solar regulators with Victron energy-smart Bluetooth controlled regulators; and 3) replacement of AGM batteries with lithium batteries. 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). 3G antenna were maintained but will require replacement in the future.

3.3.2. Status of tagged Murray cod population

In 2021, a total of 47 radio-tagged Murray cod were detected within the Chowilla Anabranch and adjacent River Murray via manual or remote tracking (Figure 3). A further seven fish have tags with remaining battery life but were not detected (last detected between July 2019 and December 2020).

Of the 54 tagged Murray cod identified above, seven have tags that remain operational despite exceeding their estimated battery life (2019), and as such, are likely to cease functioning soon. A further 17 have tags 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 ≤30 individuals.

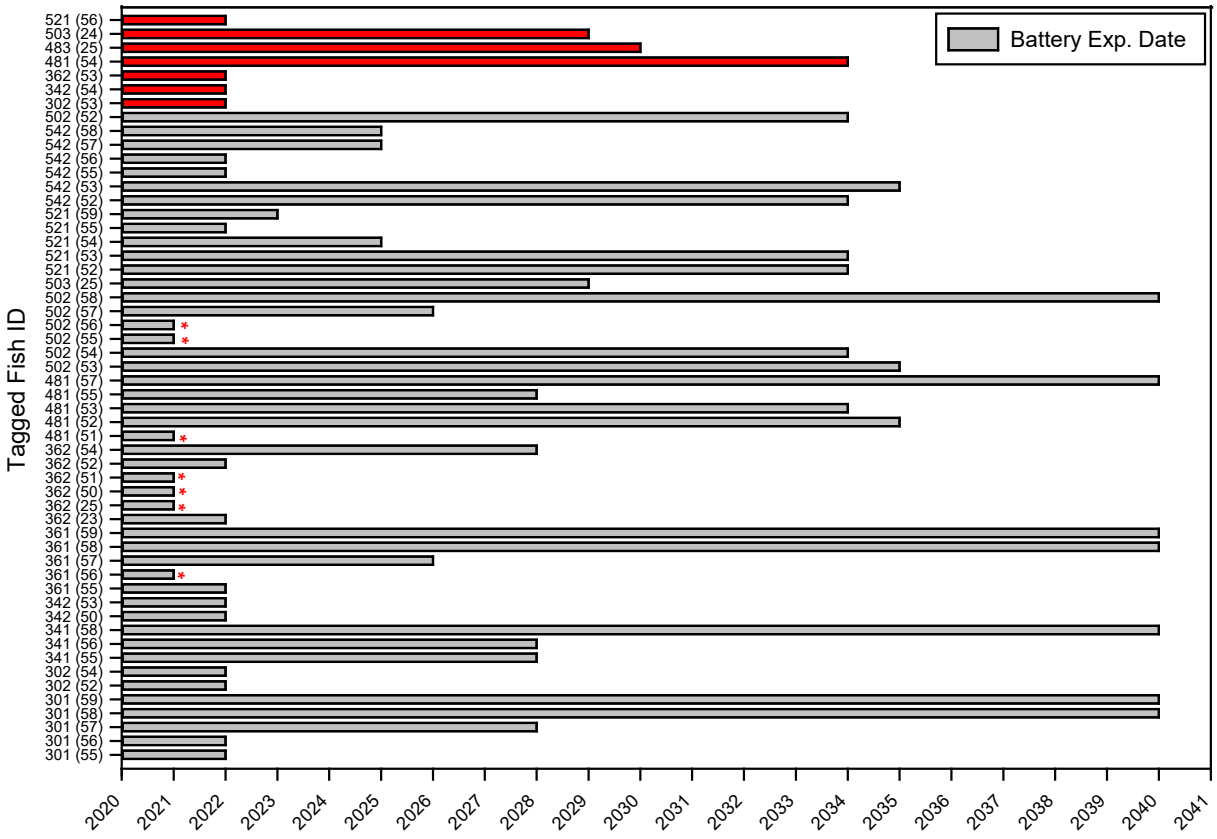

Figure 3. The expected battery-life of radio-tags fitted to 47 actively tagged Murray cod within the Chowilla Anabranch as of December 2021. The red Asterix indicates radio-tags that are still operational despite their expected battery-life expiring prior to December 2021.

4. DISCUSSION

The Chowilla Anabranch 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), the Chowilla Anabranch has been subject of a long-term fish condition monitoring program (Fredberg et al. 2021) and an associated intervention monitoring program that supports targeted investigations (e.g. Fredberg and Zampatti 2018) to inform specific interventions and adaptive management. This report presents the fish intervention monitoring program for 2020/21, which included three specific but related components, namely: 1) assessing reproductive variance and effective population size of Murray cod; 2) assessing fish movement and habitat use within Boat Creek; and 3) 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

The results of this component of the study provide further evidence of previously observed patterns within Murray cod mating systems, including the presence of within-seasonal polygamy, between-seasonal polygamy, and reproductive skew within breeding populations (Couch et al. 2020, O'Dwyer 2022). In Chowilla, one instance of repeat pairing between Murray cod breeding adults across years (inter-seasonal monogamy) was also found, suggesting in natural environments, Murray cod may exhibit mixed mating strategies (Couch et al. 2020). While only 3 breeding adults sequenced during this study were assigned to sampled offspring, each adult was likely over 10 years of age suggesting older age Murray cod may contribute significantly to successive generations. Future collection and sequencing of a greater number of reproductively mature fish will provide greater insight on contribution adults of different sizes and ages.

The 164 offspring sequenced from 2017–2021 were estimated to be derived from 216 different parents. This included a high proportion of offspring derived from unique parents (i.e. no full or half siblings were detected) and suggests that the breeding adult population within Chowilla is significantly larger than 216 adults. To reliably infer the exact number of effective breeding adults

contributing year to year, a larger sample of offspring is required. A cumulative breeding adult detection curve was developed collectively for Chowilla and the adjacent Lindsay-Mullaroo system and suggests that there may be upwards of ~3000 breeding adults across both systems (Appendix). This detection curve, however, is based on relatively few offspring (*n* = 220) and caution should be exercised when extrapolating the population size. As more annual collections are taken, the cumulative value of each new sample increases, meaning that for each subsequent sampling year, more assignments will be found, and fewer samples will be needed to achieve the same levels of accuracy. Although difficult to determine exactly how many samples would be required for each year, considering the previous detection curve, the low rate of detecting multiple offspring here, and the yearly nature of collections, a yearly sampling of 75–100 offspring compounded over ~5 consecutive years (375-500 offspring over 5 years) should enable the detection of variation in breeding adult outputs across years to indicate responses to environmental variability and management interventions.

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 represent the movement and spawning of reproductively mature individuals in both systems 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; ARI unpublished data) lending support to the first mechanism. Together, kinship analysis and radiotelemetry indicate connectivity between Murray cod populations in two perennial lotic systems in the predominantly lentic lower River Murray.

Values of N_e varied annually suggesting that some breeding seasons have significantly more breeding adults contributing to reproductive output and therefore offspring collectively have higher genetic diversity. For example, despite having the largest cohort size (i.e., number of offspring sequenced), the 2018 breeding season appeared to have half the N_e of 2017 or 2020, suggesting fewer adults contributed to that years cohort (defined as a combination of larvae and YOY). 2018 was the only year among 2017–2020 when the Chowilla Regulator was operated during the Murray cod spring spawning season and was accompanied by changes in hydraulics and the typical distribution of larvae within the system (Gibbs et al. 2020). Furthermore, recruitment to YOY in autumn 2019 was the lowest among the years 2018–2021 (Fredberg et al. 2021). Within the 2018 spawned cohort, however, one adult was found to contribute disproportionately to offspring sampled and sequenced (20), and thus, it remains unclear if low N_e from 2018 spawning

was an artefact of sampling or if operation of the regulator impacted reproduction. All N_e values derived from yearly cohorts were lower than the N_e generated across all years, suggesting that offspring sampled from any single breeding season did not represent all breeding adults contributing over the broader time period providing further evidence that breeding population size is likely considerably greater than currently estimated.

Within Chowilla, the N_e estimates calculated for each breeding season were largely correlated to the estimated ratio of breeding adults detected to offspring sequenced, with the highest N_e years aligning with the highest ratio of breeding adults per sequenced offspring. This suggests that, provided sufficient sample sizes are available year to year, N_e may provide a reliable estimate of the relative genetic diversity and number of breeding adults contributing to a recruitment event (based on offspring sampled). While this measure is not as sensitive to small fluctuations, and exact estimates of breeding adult numbers year to year, N_e may provide a rapid and easy measure to infer poor and good quality breeding seasons while requiring fewer samples than a kinship-based approach. Given the low sample sizes, and the relatively few years N_e was calculated, however, the use of N_e as described would require further validation to confirm its accuracy.

4.2. Fish movement and habitat use in Boat Creek

In 2020/21, we established an array of 26 acoustic receivers in Boat Creek and the broader Chowilla system. This receiver array is supplemented by receivers in the River Murray main channel deployed under allied projects funded by the DEW and MDBA, which will broaden the regional coverage of the current array. Within Chowilla, the array was deployed to: 1) provide fine-scale tracking of fish movement within Boat Creek (receivers approximately every 500 m); 2) assess passage and delay at the Boat Creek Bridge; and 3) assess broader scale movements among major tributaries within the Chowilla Anabranch and the River Murray.

In Boat Creek, 38 large-bodied fish (Murray cod = 7, golden perch = 17, freshwater catfish = 4 and common carp = 9) were captured and implanted with acoustic transmitters and their movements will be monitored across 2021/22 with a receiver download scheduled for June 2022. The movements of these fish will elucidate patterns of habitat use and residency in Boat Creek and the influence of the Boat Creek Bridge on connectivity. Operation of the Chowilla Regulator in spring 2021 may provide the opportunity to assess fish movement when the hydraulic step at the bridge is drowned out and contrast this to alternative head differentials at this putative barrier. This investigation will continue under Intervention Monitoring in 2021/22.

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

The spatial ecology of Murray cod in the Chowilla system 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). These studies have used radiotelemetry and been supported by a series of nine tele meted 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 2021, software was upgraded in the KLK3000 units in all logging towers, while two loggers, at the junction of Slaney and Chowilla creeks, and at the junction of Chowilla Creek and the River Murray were extensively upgraded. This included new solar panels and regulators and battery replacement. Since upgrade, these remote logging stations have been performing satisfactorily and three further stations are scheduled for upgrades under Intervention Monitoring in 2021/22. This will leave four remaining towers with original technology, yet these stations remain operational.

In 2021, a total of 47 tagged Murray cod had active tags and were detected within or adjacent the Chowilla system in via manual tracking or on remote logging stations. A further seven fish likely had active tags but had not been detected since 2020. Based on estimated battery life, a substantial number of tags will expire by end-2022 leaving approximately 30 fish with active 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. As such, the continued use of radiotelemetry to study spatial ecology of Murray cod at Chowilla and the adjacent River Murray would therefore be reliant on tagging further individuals at some stage during 2022–2024.

5. CONCLUSIONS AND RECOMMENDATIONS

In 2020/21 we investigated the feasibility of using genetic approaches to assess reproduction, movement and population size of Murray cod, to complement more traditional approaches including telemetry and condition monitoring of abundance. Molecular kinship assignment provided insights into population dynamics at Chowilla, including within and across-season polygamy, and monogamy, demographic connectivity among Chowilla and the Lindsay-Mullaroo system, and reproductive variance. A limited sampling period and low sample sizes currently limits inference, yet there appears to be inter-annual variability in the numbers of breeding adults at Chowilla. Continued sampling and sequencing will improve understanding of annual reproductive variance, the nature of connectivity between Chowilla and Lindsay-Mullaroo, overall population size and the reproductive contribution by adults based on age/size.

An investigation of fish movement in Boat Creek and further upgrades to radio-telemetry infrastructure are ongoing in 2021/22 and will be reported upon in a subsequent Intervention Monitoring Report. The results of the current project, nonetheless, highlight several future investigations and tasks to build upon past studies and further inform management of the Chowilla system, particularly regarding the ecology of Murray cod. As such, we recommend the following:

- Continuing investigations of adult contribution, movement and effective population size using genetic approaches. Interpretive power will increase with increasing sampling period and sample size providing greater insight on the influence of management on reproduction/recruitment and connectivity among Chowilla and Lindsay-Mullaroo.
- Increasing the number of radio-tagged Murray cod within Chowilla. Maintaining a tagged population (ideally ≥50 individuals) is critical to the rigour of investigations of movement and habitat use in association with management of Chowilla and the adjacent River Murray.
- Further maintenance and upgrade of radio-telemetry towers. Following Intervention Monitoring in 2021/22, all towers in immediate need of maintenance will have been upgraded with new componentry. The remaining four towers are operational, yet upgrades will be required to ensure all remote logging stations have contemporary software, power supply and management systems, and communications.
- Undertaking a meta-analysis of Murray cod movement data since 2007. To-date, investigations of Murray cod movement at Chowilla have investigated specific aspects of

movement, including movement and mortality in association with hydrology and blackwater (Leigh and Zampatti 2013), and the influence of the Chowilla Regulator on habitat use and obstruction of individual movement (Zampatti et al. 2016, Fredberg et al. 2019). An analysis of the full movement dataset (2007–2022) incorporating pre- and post-regulator periods, and a range of hydrological scenarios could provide great insight on the spatial ecology of Murray cod at Chowilla and the potential influence of management interventions.

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7. APPENDIX

Estimating the proportion of total breeding adults detected in this study

Method

To determine the likely proportion of total breeding adults within Chowilla and Mullaroo, the number of breeding adults was estimated based on each offspring's inferred parents from the BestCluster file. All offspring were randomly subsampled, and the total number of unique parents identified based on the set number of offspring sequenced, was calculated. This subsampling was repeated 99 times to generate a cumulative detection curve based on the number of offspring sampled. Using the detection curve generated, a mathematical relationship between unique breeding adult detection and offspring sampling was estimated based on the highest correlating exponent-based equation to the offspring data [e.g., y= ax +b –(cx^d), where y= number of unique breeding adults, x= offspring sequenced, and a,b,c, and d are constants]. All calculations and bootstrapping were undertaken using the R package *stats* and custom scripts.

Results

No clear pattern of detection plateauing (indicating most adults had been detected in the study system) was observed from the offspring sequenced (Figure 1). The relationship between the number of unique parents detected from a set number of sequenced offspring was found to most closely be represented by the equation; y = (3.45*x) -(x ^1.130) [correlation of 0.99998 with the original offspring data]. Extrapolating the sample sizes until approximate plateauing of new parent detections using the above equation suggests approximately 2300 offspring would be required to sample 75% of all adults, and 3600 offspring would be required to sample 95% of all adults (~ 3000 adults). As only 220 offspring were used in this study, caution should be made when trying to infer likely sample sizes as true parental detections may plateau significantly faster than predicted here once larger sample sizes of offspring are included. As sampling took place over multiple periods to maximise unique breeding adult detections, the current sampling design may lead to more unique parents observed than would be expected by random sampling, inflating the estimated breeding adult population size above.

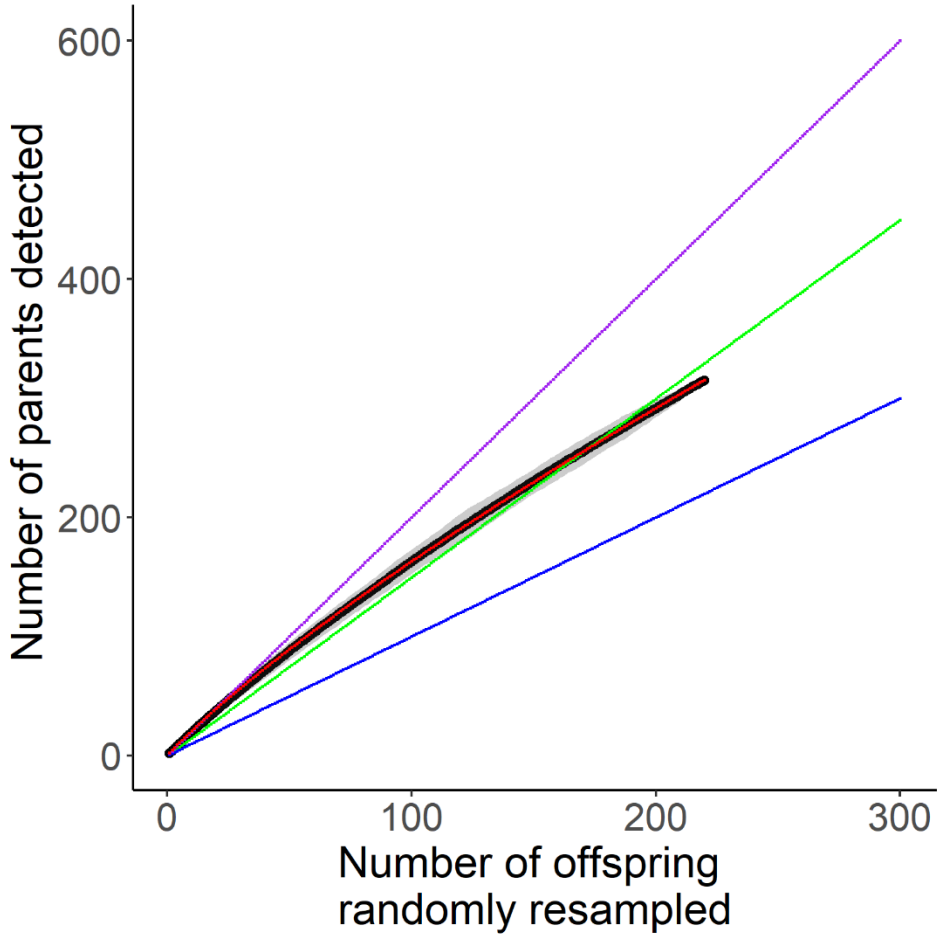

Figure 1. The number of unique parents detected per offspring sampled. Murray cod data are shown as black dots with a 95% confidence range around points shown as a grey area around each black point. The 95% confidence range was developed using 100 bootstrap replicates of the data. The purple line shows a standard 2–1 relationship where every offspring has two unique (previously undetected) parents. The blue line shows a 1–1 relationship where every offspring has only one unique parent. The green line shows a 1.5–1 relationship where every offspring on average has 1.5 unique parents. Lastly, the red line shows a relationship where the number of unique parents = (3.45*number of offspring sequenced) - (number of offspring sequenced ^1.130).